INVESTIGATION OF THE EMULSIFYING PROPERTIES OF BAMBARA GROUNDNUT FLOUR AND STARCH

By

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Thesis submitted in fulfillment of the requirements for the degree
Master of Technology: Chemical Engineering

In the Faculty of Engineering

At the

CAPE PENINSULA UNIVERSITY OF TECHNOLOGY

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Bellville Campus

December, 2013

DECLARATION

I, EBUNOLUWA GRACE GABRIEL, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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ABSTRACT

A number of foods, pharmaceutical and industrial products are formulated as emulsions. The immiscibility of oil and water makes emulsions thermodynamically unstable, thus requiring emulsifiers. Natural and synthetic substances have been used as emulsifiers with preference for the former for safety, cost and availability purposes. Bambara groundnut (BGN) is an important source of nutrient in many African communities. Similar to soybean, flaxseed, and other leguminous products, the important blend of high protein and carbohydrate composition of BGN makes it a potential candidate as an emulsifier where, it can serve additional nutritional role in such emulsions. No literature evidence exists to support any earlier study on the potential emulsifying property of BGN. It is therefore of interest to investigate the potential of BGN flour (BGNF) and starch (BGNS) in stabilizing oil-in-water emulsions.

The current study aimed to investigate the emulsifying properties of Bambara groundnut flour and starch.

A batch of BGN was milled to produce the BGNF. BGNS was extracted from the BGNF. Emulsions were prepared using a wide range of flour-oil-water and starch-oil-water composition as generated through computational modelling. Emulsions were physically observed for stability, and then subjected to various stability studies using the Turbiscan® (which gives kinetic information on the process leading to phase separation; and allows for the detection of two kinds of destabilisation phenomena (particle migration): creaming and sedimentation) and optical microscope (which helps to detect particle size variation or aggregation in order to measure coalescence and/or flocculation). The most stable emulsions (one BGNF-stabilized and the other BGNS-stabilzed) were determined and subjected to same stability studies in the presence of varying physicochemical and physic-mechanical environmental conditions: effects of homogenization speed (9,000 – 21,000 rpm), pH (2 - 10), the presence of salt (2 – 10% w/v NaCl) and vinegar (2 – 10% v/v). Results were expressed in terms of relative stability (using creaming/sedimentation rate) and absolute oil droplet growth (coalescence and flocculation).

The emulsifier-oil compositions (emulsifier and water make 100 mL gelatinized mixture before the addition of oil) that gave the most stable emulsions were 9 g BGNF-39 g oil and 5 g BGNS-30 g oil. The two emulsions had only 15% and 30% growth in oil droplet diameter respectively by day 5, compared to over 3000% in the unstable ones. The emulsions were generally observed physically to be stable till day 5 with minimal creaming. Emulsions prepared with BGNS were physically observed to have increasing viscosity with time. There was microbial growth on the emulsions after 3 days. This might have been encouraged because of the rich nutritional composition of BGN. The stability of the emulsions increased significantly ($p < 0.05$) with the speed of homogenization (up to 15, 000 rpm beyond which the difference in stability was not significant). Compared to control (pH 7), emulsion stability decreased significantly ($p < 0.05$) with decreasing (up to 16% instability by Day-5 at pH 2) and increasing pH (10% instability at pH 10) with corresponding increase in droplet size. Emulsion stability increased significantly ($p < 0.05$) with increasing NaCl concentration (35% instability at 2% NaCl compared to 18% instability at 10% NaCl). Stability however decreased sharply with increasing concentration of vinegar (55% instability at 10% vinegar compared to 19% instability at 2% vinegar). The BGNF-stabilized emulsions were more stable (ordinarily and in the presence of the additives) than the BGNS-stabilized ones. Instability values in the BGNS emulsions, in most cases, double those for BGNF.

Emulsions were successfully stabilized with BGNF and BGNS. The emulsions were optimal when prepared at a homogenization speed of 15,000 rpm and at neutral pH. The higher the concentration of salt, the more stable the emulsions. Increasing concentration of vinegar however, destabilized the emulsions.

ACKNOWLEDGMENTS

I will like to express special gratitude to my supervisors, Prof Daniel Imwansi Ogiemwenya Ikhu-Omoregbe and Prof Victoria Jideani for providing the invaluable opportunity to carry out this research. I am most grateful for their suggestion, support and guidance throughout the research period.

I will like to thank the staff and students of the departments of Chemical Engineering, and Food Technology of the Cape Peninsula University of Technology for their support and encouragement.

I am indebted to my husband, Dr Pius Fasinu for his understanding and willingness to always help. I will not do without appreciating the gift that God gave me in the course of the program, my baby boy Setonji JesuVivi Fasinu, for ‘co-operation and understanding’ that ‘mummy must go to work’.

Special thanks to my beloved parents Mr and Mrs S D Gabriel for laying the foundation on which this achievement is being built. I appreciate the moral support of my brothers and sisters – Hannah, Funmilayo, Seimo, Yenukunme, Oluwanfunke, Remilekun, Ebenezer, Semawon, Pesu and Oluwatobi. May God bless you all.

I am also grateful to my senior colleagues for their invaluable assistance. I am particularly thankful to Mr Adeyi Oladayo for his support and ideas; Dr SO Olatunji for his help during the critical stage of my result interpretation; Dr (Mrs) Elizabeth Aransiola for all the encouragement and motivation; and Mr and Mrs Samuel Egieyeh for their friendly assistance and generous help on every occasion.

And finally, to Him, the ever faithful God, my very present help in times of need, I return all the praise, honor and adoration. Without Jesus, I am nothing. I am grateful.

DEDICATION

To my loving little son, Setonji Jesuvivi

May God bless and keep you

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CHAPTER ONE

Motivation for the Study

1.1 Background of the Research Problem

Bambara groundnut [BGN] (*Vigna subterranea*), a leguminous plant which belongs to the family fabaceae is an indigenous Africa plant that has been cultivated all over sub-Saharan Africa (Mpotokwane et al., 2008). As important as BGN is as a source of food in many African communities, very limited studies have been conducted to assess its potential benefit to the food and pharmaceutical industries. Its high protein and carbohydrate content offers an important blend for commercial as well as domestic applications. One of the possible ways to employ BGN commercially is in the emulsification process.

An emulsion is formed by dispersing one liquid phase (called the dispersed or internal phase) in another (called the continuous or external phase) in which it is immiscible. The dispersed phase of emulsions is usually in fine droplets (McClements, 2005). The molecules of the two phases of emulsions are in direct contact at the interface. This makes the system thermodynamically unstable with high tendency for phase separation (Guzey et al., 2004). Homogenization, a process of high speed blending that breaks and suspends the internal phase into the continuous phase, increases the entropy of the system, but is not enough to counter the unfavorable increases in enthalpy due to the contact between the hydrophilic and hydrophobic molecules (Walstra, 1993). The result is an unstable dispersed system due to the presence of repelling interfacial forces acting between the molecules of the two liquids. Hence, the preparation of emulsions requires the addition of exogenous surfactants otherwise called emulsifying agents or emulsifiers through a process known as emulsification. The emulsifiers adsorb at the liquid-liquid interfaces thus, reducing the enthalpy contribution to the free energy. This phenomenon reduces the tendency for phase separation (McClements, 2005). The goal of emulsification is to maintain the initial state attained after homogenization of two immiscible liquids.

Emulsification is important in the food, pharmaceutical, cosmetic and chemical industries where it is applicable in formulation of products requiring simultaneous presence of aqueous and oil phases. Examples of pharmaceutical emulsions are liniments and some liquid multivitamins incorporating both oil- and water-soluble vitamins while cosmetic emulsion preparations include lotions, creams and ointment. Emulsification is also applied in the preparation of various food products including mayonnaise and margarine preparations. Paints and other color preparations are examples of industrial application of emulsions (Leal-Calderon et al., 2007; Sikora et al., 2008; Grigoriev and Miller, 2009).

In order to establish the suitability of a prospective emulsifier for emulsion preparation, various stability tests are required. The choice of adequate emulsifying agent is critical in the preparation of stable emulsions. The most important ingredient for the preparations of stable emulsions is the emulsifiers. As a result, various compounds have been investigated for suitability as emulsifiers. Emulsion stability is the ability to maintain the initial state of dispersion attained after homogenization of the two immiscible liquid. Ideal stability tests model stress to be encountered in the life span of the emulsion. Thus, most emulsion stability tests involve the monitoring of emulsion properties under varying temperature, pH and other physicochemical conditions over a period of time. Since emulsions are meant to have long shelf lives, ideal stability tests should be performed over similar time length. This is difficult. However, technological advancement has made it possible to analyze potential instability within a short time post-preparation (Leal-Calderon, 2007).

1.2 Problem Statement

There is growing interest by the food, pharmaceutical and cosmetic industries in replacing synthetic emulsifiers with natural ones. This is because natural emulsifiers are more biocompatible and could demonstrate satisfactory amphiphilic properties (Nakauma et al., 2008; Wang et al., 2010). The search for such natural products has yielded promising results with the use of some leguminous plant products like soybean, casein, flaxseed, polysaccharides among others in emulsion preparations (Huck-Iriart et al., 2011).

The nutritional composition of BGN flour and starch indicates its potential as an emulsifier. However, not much is documented about its potential as an emulsifier. It is therefore of interest to investigate the potential of BGN flour/starch in stabilizing oil-in-water emulsions.

1.3 Broad Objective

The aim of this study was to investigate the emulsifying properties of Bambara groundnut (BGN) flour and starch in oil-in-water emulsion.

1.3.1 Specific objectives

The specific objectives include to:

i. Determine optimal concentration of BGN flour/starch required to stabilize typical oil-in-water emulsion.

ii. Establish the effect of stress conditions (homogenization speed, pH, salt (NaCl) and vinegar) on the stability of the BGN flour/starch-emulsified emulsions over a period of time.
iii. Investigate the effect of time on the storage stability of emulsions stabilized with BGN flour and starch.

1.4 Hypotheses

It was hypothesized that:

1. BGN flour/starch acting as an emulsifying agent is capable of stabilizing oil-in-water emulsion preparations.
2. BGN stabilized emulsion will be stable to homogenization speed up to 21000 rpm, pH (2 to 10), vinegar (2 to 10%) and salt (up to 0.2 M sodium chloride).
3. Storage time will have an effect on the BGN stabilized emulsion.

1.5 Delineation of the Research

The study does not intend to conduct phytochemical analysis of BGN flour/starch. It is beyond the scope of the current study to assess the safety or otherwise of the products extracted from BGN in living cells/organisms. The study is also limited to the stated variety of BGN.

1.6 Significance of the Research

This research is important in the continuous search for ideal emulsifying agents. It is particularly promising because BGN can be used traditionally as food and medicinal product. It is a cheap source of protein and useful outcome from this study can easily be applied in the food/pharmaceutical sector. Current knowledge on BGN and its emulsifying ability is sparse. The finding from this study will significantly add to the body of scientific knowledge concerning BGN. It will provide data for potential use of BGN in food, pharmaceutical and industrial emulsions. The findings from this study will also provide the basis for further studies on BGN especially to nutritionists and food scientists.

1.7 Expected Outcomes and Potential Contributions of the Research

Findings from the current study will be presented in international/national conferences and at least one article will be published in peer-reviewed accredited academic journal. The outcome of the study can also increase the economic value of this BGN crop.

1.8 Keywords

Emulsion, emulsifier/emulsifying agent, Bambara groundnut, emulsion stability, emulsion breaking, creaming, coalescence, flocculation, internal/external phase, dispersion

CHAPTER TWO

Literature Review

2.1 Overview of Emulsion

An emulsion is formed by dispersing one liquid (called the dispersed or internal phase) in another (called the continuous or external phase) in which it is immiscible. The two immiscible liquids involved in industrial emulsions especially in food industry are often oil and water, although this may not always be the case as "water-in-water" emulsions have been produced (Norton et al., 2001; 2006; Simon et al., 2007).

The dispersed phase of emulsions is usually in fine droplets. The size of the dispersed globules is often used to classify emulsions as microemulsion (0.01 to 0.1µm) or macroemulsions (up to 5µm) (McClements, 2005). In emulsified food products, the average droplet diameter typically falls in the 0.1 - 100 µm range. The relative spatial distribution of the phases is used to classify emulsions into oil-in-water (O/W) or water-in-oil (W/O). Thus, an emulsion made of oil droplets dispersed in an aqueous phase is referred to as an oil-in-water emulsion which is the object of this project. However, water droplets dispersed in an oil phase is referred to as a water-in-oil emulsion. Examples of O/W emulsions include milk, cream, ice-cream, dressings, mayonnaise, beverages, soups, dips and sauces. Typical W/O emulsions are butter, margarine vinaigrette and topical ointments (Figure 2.1). Other forms of emulsion have been expressed as multiple emulsions in which case smaller droplets are contained in the dispersed phase producing such emulsion types as oil-in-water-in-oil (O/W/O), water-in-oil-in-water (W/O/W) or oil-in-water-in-water (O/W/W) (Garti and Bisperink, 1998; Friberg et al., 2004; Kim et al., 2006).

2.2 Preparation of Emulsion

Homogenization is the process of converting bulk oil and bulk water into an emulsion, or of reducing the size of the droplets in an already existing emulsion. It is usually achieved by the application of intense mechanical (manual or automated) agitation to the liquid mixture (Walstra, 1993; 2003). It is a process of high-speed blending that breaks and suspends the internal phase into the continuous phase, thus increasing the entropy of the system (Figure 2.2). This however is not enough to counter the unfavourable increases in enthalpy due to the contact between the hydrophilic and hydrophobic molecules (Walstra, 1993). The result is an unstable dispersed system due to the presence of repelling interfacial forces acting between the molecules of the two liquids. Hence, the preparation of emulsion requires the addition of exogenous stabilizers otherwise called emulsifying agents or emulsifiers through a process known as emulsification (Figure 2.3).

Figure 2.1 Examples of emulsion formulations encountered in everyday life: A) margarine, B) asphalt, C) pesticides, D) ointment, E) paints, F) metal cutting oils, G) ice cream, H) skin creams and I) mayonnaise

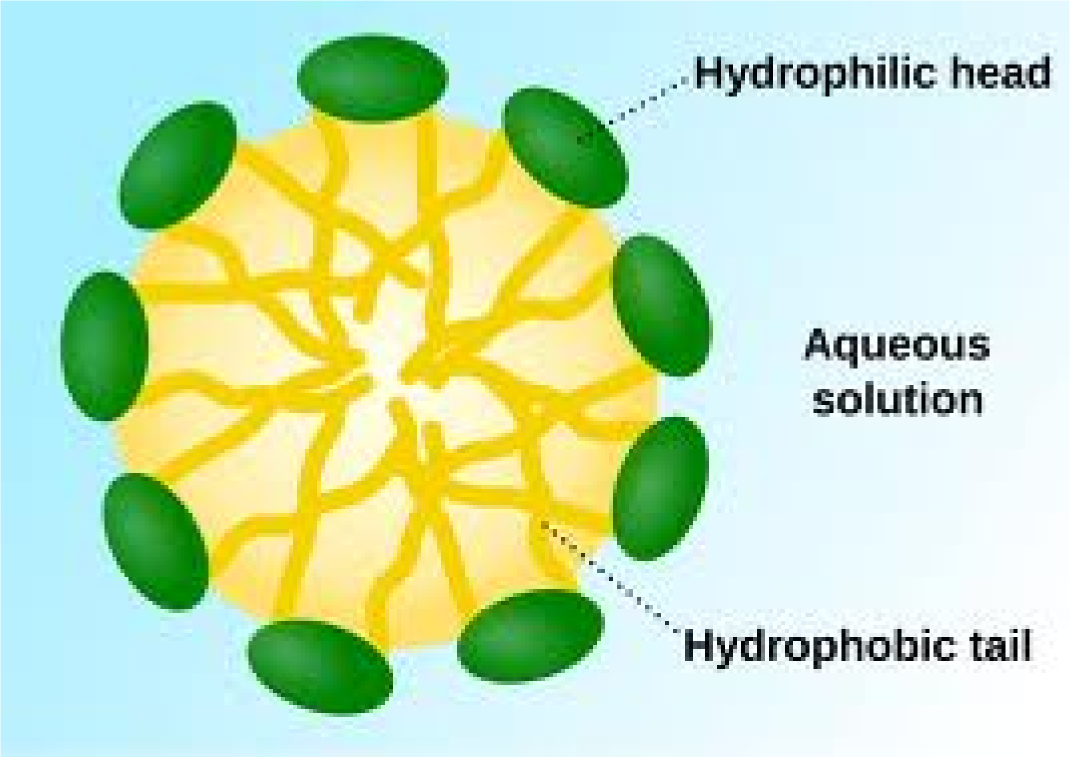


Figure 2.2 Principle of emulsification: figure showing oily droplet suspended in aqueous continuous phase by the hydrophilic-hydrophobic portions of emulsifier molecules (Source: http://www.gobiobased.com/Surfactant.html)

The emulsifiers adsorb at the liquid-liquid interfaces thus, reducing the enthalpy contribution to the free energy. This phenomenon reduces the tendency for phase separation (Charcosset, 2009). The goal of emulsification is to maintain the initial state attained after homogenization of two immiscible liquids (McClements, 2005). In the food industry, emulsions that are kinetically stable for days, or weeks, months or even years require the incorporation of emulsifiers.

As surface acting agents, emulsifiers must possess good surface activity and the ability to form a condensed interfacial film (Guzey et al., 2004). Various theories of emulsification have been proposed in order to explain the stabilizing effects of emulsifiers (McClements, 2005). The surface tension theory explains emulsification as the lowering of the interfacial tension between the aqueous and the oily phase leading to a reduction in the repellant forces between the two liquids and a decrease in the attraction between the molecules of the same liquid. The oriented-wedge theory proposes the formation of mono-molecular layer of the emulsifying agents which is curved around the droplets of the dispersed phase. The interfacial film theory explains emulsification as the prevention of contact and coalescence of dispersed phase due to the formation of film of emulsifying agents which stabilize the emulsion (McClements, 2005). In addition to the ability to hold together the dispersed phase in the continuous phase, an ideal emulsifier must be a stable material or compound, be non-toxic, must not inflict unacceptable colour, taste or odour on the emulsion system, and must be compatible with other components of the preparation (McClements, 2005).

In addition to emulsifiers, other stabilizers that play important roles in emulsion stability are called texture modifiers (Akhtar, 2005). While emulsifier are surface-acting, texture modifiers either increase the viscosity of the continuous phase (in which case they are called thickening agent or thickener) or form gel network within the continuous phase (where they are referred to as gelling agent), leading to a decrease in droplet movement due to gravity or Brownian motion (Quintana et al., 2002). Various types of polysaccharide and protein ingredients have been used as thickening or gelling agents in food emulsions, including starch, modified starch, cellulose, modified cellulose, pectin, alginate, carrageenan, gelatine, whey, protein, caseinate, soy protein and egg protein (Benichou et al., 2002). It is not known yet the mode of action for BGN flour and starch in stabilising emulsions.

2.3 Characterization of Emulsions

A number of standardized procedures are generally applied in the determination of instabilities in emulsions. Parameters often characterized are those around the dispersed droplets and their interfacial behaviour.

Figure 2.3 Commonly used instrument for mechanical agitation of immiscible liquids to form emulsions: A) colloid mill for industrial preparation; B) pestle and mortal; and C) mechanical stirrers for small scale emulsion preparations

2.3.1 Droplet concentration

The concentration of droplets in emulsions can be characterized in terms of the volume fraction (φ) of the dispersed phase. This is calculated by dividing the volume (V_d) of the emulsion droplets by the total emulsion volume (V_t). For practical purposes however, droplet concentration is more conveniently expressed in terms of dispersed phase mass (φ_M) which is defined as the mass of emulsion droplets (m_D) divided by the total mass of the emulsion (m_E). The relationship between these variables is provided in the following equations (McClements, 2007):

$$\phi_M = \phi[\phi + (1-\phi)\frac{\rho 1}{\rho 2}]^{-1} \qquad \text{Equation 1}$$

$$\phi = \phi_M[\phi_M + (1-\phi_M)\frac{\rho 1}{\rho 2}]^{-1} \qquad \text{Equation 2}$$

In equations 1 and 2 ρ1 and ρ2 are the respective densities of the continuous and the dispersed phase. From the equations, when the dispersed and continuous phases have the same density, the mass fraction will be equivalent to the volume fraction. It should also be noted that droplet concentrations can be expressed as either a dispersed phase volume percentage ($\varphi\% = 100 \times \varphi$) or disperse phase mass percentage ($\varphi_M\% = 100 \times \varphi_M$). In this study, the density of the dispersed phase (oil) is 0.91 g/ml and the continuous phase is 1 g/ml hence it is expected that the mass fraction of this emulsion will not be equivalent to the volume fraction since the density of the dispersed and continuous phase are different.

2.3.2 Droplet size of emulsions

The size of the dispersed droplets has strong influence on the stability of emulsions. Thus, emulsion instability increases with increasing droplet size. The size of emulsion droplets can influence flocculation and coalescence, gravitational separation (creaming or sedimentation), optical properties like color and transparency, rheological characteristics including viscosity; and sensory feel (Robins et al., 2002).

An emulsion is said to be monodispersed when the droplet sizes are uniform while polydispersed emulsions have droplets that vary in size. Polydispersed emulsions are the most common in practice because most emulsions contain wide ranges of droplet sizes. The sizes of emulsion droplets are usually measured as the radius or diameter. Since most emulsion droplets sizes vary, it is common to express droplet sizes as ‘droplet size distribution’ defining the concentration of droplets in different size classes (Huang et al., 2001). Thus, droplet size distribution is commonly and conveniently presented as tables or histograms presenting the size classes and their concentration (Walstra, 2003). Graphs of such size distribution can be plotted as mid-point diameter or mid-point radius against the volume percentage or number percentage of the droplets within the particular size class.

According to the method of Krstonošić et al. (2009), the particle size of prepared emulsions can be characterized employing light microscope. The droplet size distribution is determined from the microphotographs. Emulsion samples are observed with an optical microscope equipped with camera. Counted droplets are classified into size classes and the distribution determined by equations 3 and 4.

$$d_n = \frac{\sum n_i d_i}{\sum n_i}$$ *Equation 3*

$$\sigma = \frac{\sum n_i (d_i - d_n)}{\sum n_i}$$ *Equation 4*

Where n_i is the number of droplets in each size class, d_i is the droplet diameter, $\sum n_i$ is the total number of droplets, d_n is average droplet diameter, and σ the standard deviation.

2.3.3 Droplet charge

Emulsion droplets usually have electrical charges on their surfaces due to the molecules they have adsorbed. Such molecules include small ions, phospholipids, ionic emulsifiers, proteins and certain polysaccharides (Mun et al., 2005). The type and concentration of ionized surface charges on the emulsion droplets determine their electrical characteristics and their interaction with other charged species in the emulsion system (Leunissen et al., 2007). In many food emulsions, this principle is manipulated by using certain ionic emulsifiers that can adsorb to the droplet surface thereby prevent droplet aggregation by electrical repulsion (Philip et al, 2002; Dickinson, 2003; Friberg et al., 2004). The three main parameters used to characterize the electrical characteristics of a droplet are surface electrical potential, surface charge density and/or surface charge potential (Joanicot and Ajdari, 2005).

The amount of energy necessary to bring charges from an infinite distance to the surface through the surrounding medium, and thus raise the surface charge density from zero to a given value is called the surface electrical potential (Roland et al., 2003). The surface electrical potential depends on the ionic composition of the surrounding medium.

The amount of electrical charge per unit surface area is referred to as the surface charge density. It depends on the net number of charged groups per surfactant molecule and the number of adsorbed surfactant molecules per unit interfacial area (Stein et al., 2004).

The surface charge potential (also known as the zeta potential) is the electrical potential at the "shear plane," which is defined as the distance away from the droplet surface below which the counter ions remain strongly attached to the droplet when it moves in an electrical field (Rabinovich-Guilatt et al., 2004). It provides a more practical representation of the electrical characteristics of an emulsion droplet because it accounts for the adsorption of any charged counter ions. It is also easier to measure (Rabinovich-Guilatt et al., 2004).

2.3.4 Interfacial properties

The narrow interface surrounding each emulsion droplet containing the mixture of oil, water and emulsifier molecules can make up a significant fraction of the total volume of the emulsion if the droplet size is less than 1 µm (Arditty et al., 2005; McClements, 2005). The droplet interface may also influence the sensory (texture, transparency, viscosity) and physicochemical properties of emulsions. Factors that influence the properties of the interfacial region include the type and concentration of the surfactants used, physicochemical interactions within the emulsion (competitive adsorption, complexation, layer formation) and the presence or absence of electric charge (Bos and van Vliet, 2001; Dickinson, 2003). The stability of emulsions and their susceptibility to gravitational separation, coalescence and flocculation are influenced by the physicochemical properties of the interfacial region. In addition, the thickness and the rheological characteristics of the interfacial region determine the rate of movement of molecules into or out of the droplets (Dickinson, 2003; Arditty et al., 2005).

2.3.5 Colloidal interactions

The interactions between the droplets determine the propensity for aggregation and subsequent emulsion instability (Chanamai and McClements, 2000c; McClements, 2005). Colloidal interactions influence the creaming stability and rheology of emulsions. Various types of interaction between emulsion droplets include steric, electrostatic, van der Waals, depletion, hydrophobic and hydration interactions (Perrin, 2000). In addition, colloidal interaction may be classified as attractive or repulsive; weak or strong; and/or short or long based on set parameters. In general, when interactions are predominantly attractive, droplets tend to aggregate compared to repulsive interactions where individual droplets remain (Moreau et al., 2003).

Interaction potential is the energy necessary to bring two emulsion droplets from an infinite distance apart to a surface-to-surface separation. The total interaction potential is made of the contributions from the many types of interactions (Dickinson, 2008).

2.4 Stability and Instability of Emulsions

Emulsion stability refers to the ability of an emulsion to maintain its physicochemical properties overtime (Dickinson et al., 2009). The direct interfacial contact between the molecules of the two phases of emulsions however, makes the system thermodynamically unstable with high tendency for phase separation (Guzey et al., 2004). The mechanisms of emulsion instability which are often interrelated include gravitational separation (creaming/sedimentation), flocculation, coalescence, Ostwald ripening and phase inversion (Guzey et al., 2004) as depicted in Figure 2.4. These mechanisms are discussed in detail in the sections 2 4.1 to 2.4.4.

Various physicochemical factors that can influence emulsion instability include pH and pressure changes, temperature variability, relative composition of emulsion, particle size, microstructures, microbiological and environmental stresses like agitation and light. These factors may manifest at any of the preparation, storage or utilization stages (Guzey et al., 2004). It is thus important to assess how much BGN flour and starch emulsion can withstand these instability factors.

2.4.1 Gravitational Separation

Gravitational separation involves the movement of droplets as a result of density difference between the dispersed and the continuous medium. Upward movement, also referred to as creaming occurs when the droplets have lower density than the surrounding medium while downward movement called sedimentation results from higher droplet density compared to the surrounding fluid (Chanamai and McClements, 2000b). Gravitational separation is perhaps the most common mechanism of emulsion instability frequently encountered. The differences in the densities of emulsion droplets and the surrounding liquid create a net gravitational force which acts on the droplets (Robins, 2000). For example, creaming, the upward movement of the droplets occurs when the density of the droplets is lower than the density of the surrounding liquid. On the other hand, when the density of the droplets is higher, sedimentation occurs. Since the density of water is higher than that of most edible oil, sedimentation is more common in water-in-oil emulsions while creaming tendency is higher in oil-in-water. It is however, possible to

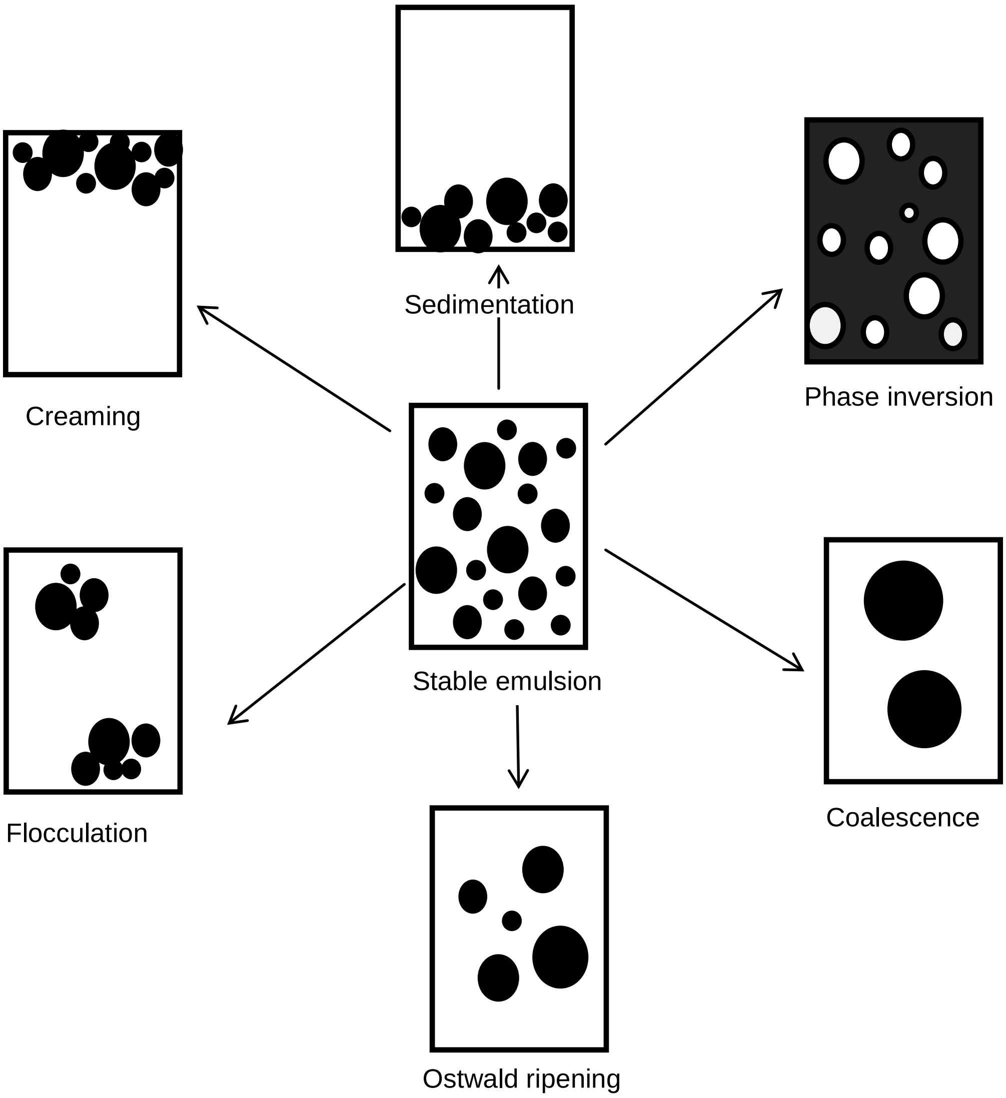


Figure 2.4 The various mechanisms of emulsion instability (Guzey et al., 2004)

have oil droplets sediment in oil-in-water emulsion if the density of the oil is increased by the presence of fat crystals or oil-soluble additives. Instances of creaming water droplets can also occur in such cases where the density of the continuous oil phase has been significantly increased (Robins, 2000). Hence, creaming was anticipated in this study because the dispersed oil phase was is less dense than the continuous aqueous phase.

One of the common ways of predicting the stability of an emulsion to gravitational separation is the use of mathematical model described by Stoke's Law. According to this law, the rate (v) at which an isolated rigid spherical droplet particles creams/sediments in an ideal liquid environment is directly proportional to the particle size (measured as particle radius r) and the density difference between the particle and the medium ($\rho_2 - \rho_1$); and inversely proportional to the viscosity (η_1) of the medium (McClements, 2005) as shown in equation 5:

$$v = \frac{2gr^2(\rho_2 - \rho_1)}{9\eta_1} \qquad \textit{Equation 5}$$

where g is the acceleration due to gravity.

A positive v indicates creaming while a negative value connotes sedimentation. Thus, to reduce the rate of gravitational sedimentation, the values of the parameters in Stoke's law can be manipulated.

Reasonable emulsion stability can therefore be achieved by decreasing the size of droplets, reduction in the density difference between the dispersed and continuous phases, or by increasing the viscosity of the continuous phase. Limitations to the application of this law include the non-homogeneity of the droplets (polydispersity), droplet concentration, interfacial interactions between the droplets, charges, interfacial thickness and non-Newtonian fluid behaviour of the continuous face (Tadros, 2004).

Determination of gravitational separation: mathematical prediction

It is possible in principle to predict the long-term stability of emulsions to gravitational separation from the knowledge of their physicochemical properties based on Stokes' Law. This can be achieved by determining the values of the parameters in Stoke's equation – the dispersed and continuous phases, the droplet size and the viscosity of the medium (McClements, 2005).

The parameters can be estimated using laboratory techniques. The density of the phases can be determined using hydrometers, density bottles, or oscillating U-tube density meters (Cunha et al., 2008). Microscopy, light scattering, electrical pulse counting or ultrasonic spectrometry methods can be used to measure the droplet sizes while viscometer or dynamic shear rheometer can be used to measure the viscosity of the continuous phase (Rao, 1999).

The complexity inherent in most emulsions may render this approach non-practical. Factors such as changes in droplet sizes over time (due to Ostwald ripening, flocculation or coalescence), polydispersity and the thickening effects of emulsifiers which influences the physicochemical properties of the phases, are responsible for the limited applicability of the mathematical models in predicting emulsion stability to gravitational separation (Cunha et al., 2008).

Determination of gravitational separation: visual observation

Visual observation is the simplest, cheapest and easiest way of monitoring gravitational separation of emulsions (Gu et al., 2005; Guzey and McClements, 2006; Ogawa et al., 2003; Velez et al., 2003). Visual observations are best performed by placing the emulsion to be analyzed in transparent tubes, agitate gently for homogeneous dispersal and left for some time or exposed to varying environmental conditions. The visual observation of the heights of any boundaries formed between the oily and aqueous layers can be measured. The extent of gravitational separation can then be determined as creaming index (CI) according to equation 6.

$$CI=\frac{H_c}{H_t}\times 100 \qquad \text{Equation 6}$$

where, H_c is the height of the creamed layer and H_t the total height of the emulsion.

Since creaming increases with time, the values of CI can be determined over an extended period of time until CI is constant (when all the droplets are closely packed into the cream layer). Graphs profiling the CI against time for different emulsion preparations will thus provide relative indication of comparative stability over time.

There are few important limitations to the use of visual observation to determine emulsion stability. It is difficult to locate the boundaries between the cream layer and the continuous phase in some emulsions for example, in very viscous or thick emulsions, and in emulsions that are colored. The extent of droplet creaming in actual droplet concentrations cannot be determined and variations in test tube sizes make CI relative and not absolute terms (Sun et al., 2007)

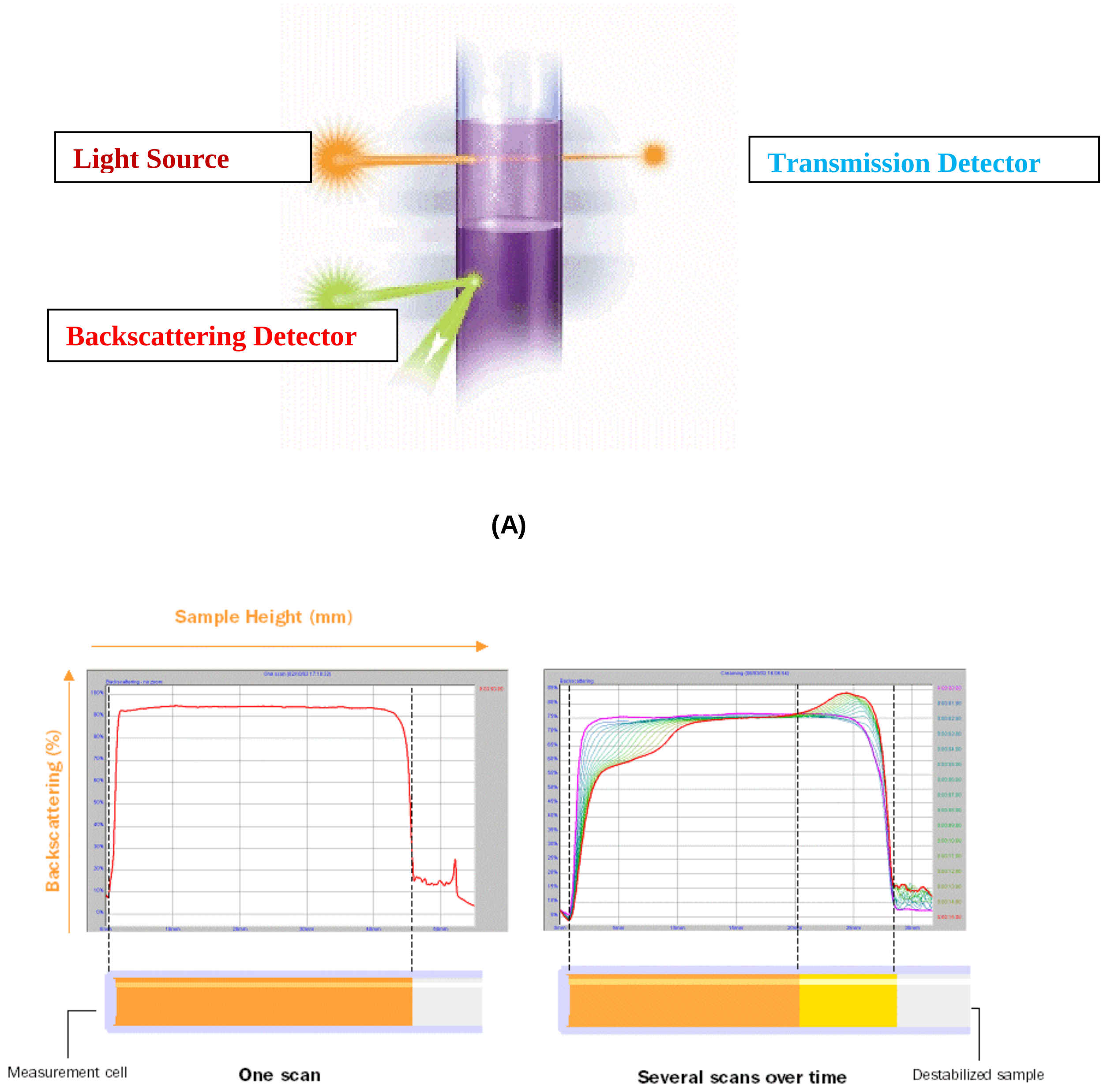
Droplet Profiling

A number of instrumental methods have been developed to characterize the physicochemical properties of emulsions. Such instruments are employed to extract information on the change in droplet concentration, and size with time. The most commonly used instrumentations are based on the principle of light scattering in which a monochromatic beam of near infrared light is directed through an emulsion placed in a vertical flat-bottomed glass tubes (Chanamai and McClements, 2000a; 2000b; 2000c). Thus, the percentage of transmitted or backscattered light measured as a function of the height of the emulsion provides an indication of changes in droplet concentrations along the height and can be used to determine creaming and/or sedimentation (Mengual et al., 1999a; 1999b). Due to the commercial availability of fully-automated analytical instruments based on this principle, optical profiling technique is one of the very popular ways for industrial characterization of gravitational separation in emulsions before such instability is visible to the eye (Mcclements, 2007).

One of such automated instrument is Turbiscan® which was used in this research work to study the kinetics of droplet movement. Turbiscan® helps in detecting emulsion destabilisation up to 50 times faster than the naked eyes (Lemarchand et al., 2003). The heart of the optical scanning analyser, Turbiscan®, consist of a detection head, which moves up and down along a flat-bottom cylindrical glass cell. The detection head is composed of a pulsed near infrared light source (λ = 880 nm) and two synchronous detectors. The transmission detector (at 180°) receives the light, which goes through the sample, while the backscattering detector (at 45°) receives the light scattered backward by the sample (Figure 2.5). The detection head scans the entire height of the sample, acquiring transmission and backscattering data every 40 µm. The Turbiscan® makes scans at various pre-programmed times and overlays the profiles on one graph in order to show the stabilization or destabilization of the emulsion (Figure 2.6). Hence, the kinetics of destabilization can be evaluated as shown in Figure 2.7. Graphs are usually displayed in reference mode, whereby the first profile is subtracted to all other profiles, in order to enhance variations. A stable product has all the profiles overlaid on one curve while an unstable formulation shows variations of the profiles (Figure 2.6). Backscattering and/or transmission fluxes are shown in ordinate and the height of the cell in abscissa (Mengual et al., 1999).

Accelerated stability tests

Accelerated study of the stability of an emulsion to gravitational separation is often performed in order to predict the long-term stability of an emulsion. This involves measurements made over relatively short times to extrapolate long-term effects. Accelerated creaming/sedimentation instability of an emulsion can be performed by centrifuging an emulsion over a predetermined period of time at a fixed speed, followed by visual observation or droplet profiling to watch for gravitational separation (Bjerregaard et al., 2001).



(A)

(B)

Figure 2.5 (A) Measurement principle and (B) Backscattering profiles of Turbiscan® (Source: Turbiscan® Manual, Turbiscan® MA 2000, Formulaction, Toulouse, France).

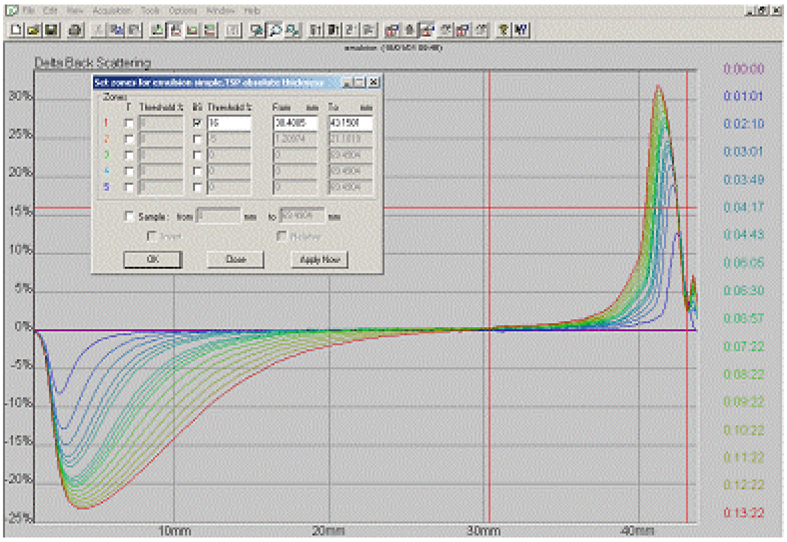


Figure 2.6 Turbiscan® profile of emulsion showing the peak thickness of creamed phase of emulsion (Source: Turbiscan® Manual, Turbiscan® MA 2000, Formulaction, Toulouse, France)

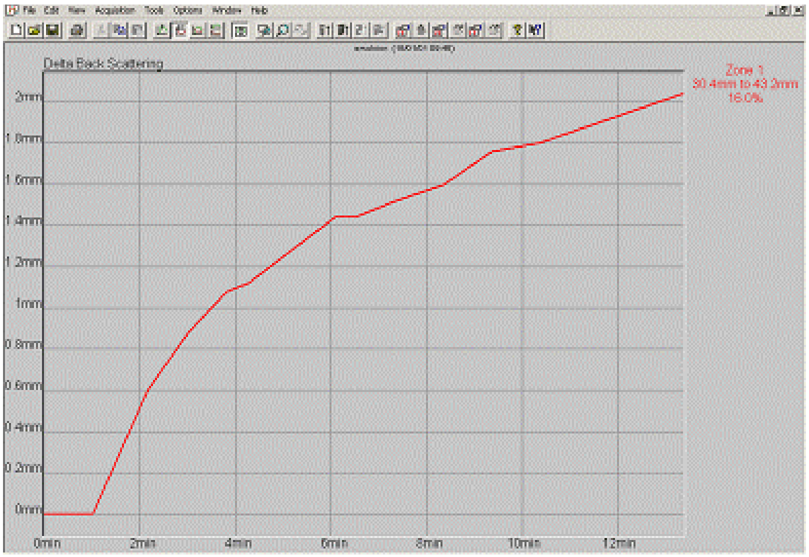


Figure 2.7 Zoomed portion of the linear part of the peak kinetics of creamed phase of emulsion (Source: Turbiscan® Manual, Turbiscan® MA 2000, Formulaction, Toulouse, France)

It is often advisable to treat the results of accelerated instability studies with caution because centrifugation does not necessarily represent the totality of the factors that emulsions are exposed to in storage. In additional, the stability of emulsions to accelerated stability studies may be misleading as other environmental stress factors outside of agitation may precipitate instability during storage (McClements, 2007).

2.4.2 Flocculation

Flocculation is the process of two or more droplets loosely stick together to form an aggregate while each droplet maintains its initial individual integrity (Vingerhoeds et al., 2005). Flocculation occurs when the forces of attraction between droplets appear to dominate over repulsive forces (Vingerhoeds et al., 2005). This makes the droplets come in close proximity to each other (flocculate), but not close enough to merge into each other (coalesce). Droplet flocculation affects the appearance, rheology, texture and general quality of emulsions. It can lead to increase in particle size which facilitates gravitational separation (McClements, 2000). It may lead to a non-uniform textural thickness especially in concentrated emulsions (Quemada and Berli, 2002).

The balance of the attractive and repulsive forces acting between the droplets creates the tendency for flocculation especially when the attractive forces are stronger. Forces of attraction responsible for flocculation in emulsions include van der Waals, depletion and hydrophobic forces while electrostatic and steric forces are responsible for inter-droplet repulsions (Chanasattru et al., 2007; McClements, 2005). Two important parameters used to characterize the rate at which droplet flocculation occurs are droplet-droplet collision frequency and collision efficiency (McClements, 2005).

Collision frequency describes the number of droplet collisions per unit volume of emulsion per unit time (Chanasattru et al., 2007). The mechanism responsible for droplet movement in the emulsion system determines the collision frequency. Such mechanisms include Brownian motion, applied mechanical forces, and/or gravity. In dilute emulsions, Brownian motion is the major mechanism for droplet movements. In emulsions with large droplets, applied mechanical forces are the major stimulant of droplet movement (Chanasattru et al., 2007; McClements, 2005). In emulsions containing large droplets with significant difference in density from that of the surrounding medium, gravitational separation are often responsible for droplet collisions. On the other hand, collision efficiency is the fraction or percentage of droplet-droplet contacts that result in actual flocculation (McClements, 2007). The collision efficiency depends largely on the droplet interaction potential which is a function of the attractive forces and distances between the droplets (McClements, 2000). If the attractive forces are weak, flocs are soon disrupted due to Brownian motion. If the forces of attraction are strong however, flocculation may lead to coalescence (Sarker, 2005). The tendency for flocculation and separation of flocs is influenced by other factors such as the size of the droplets; interfacial characteristics like charge, thickness, density, hydrophobicity; conditions of the surrounding medium of the droplets including the pH, ionic strength, temperature, osmotic pressure; and phase properties - refractive index and dielectric constant (Chanasattru et al., 2007).

Characterization of flocculation

Flocculation can be characterized by: (1) extent of flocculation, (2) flocculation rate, (3) floc size and shape, (4) Floc strength and breakage, and (5) Droplet flocculation

1 Extent of flocculation

One of the most important parameter necessary to estimate the extent of flocculation in an emulsion is the number of droplets present as monomers, dimers, trimmers, tetramers and other multimers. Thus, extent of flocculation is defined as the fraction of droplets that are contained within flocs according to equation 7 (McClements, 2007):

$$F = 100 \times \frac{N_F}{N_T} = 100 \times \frac{N_T - N_{NF}}{N_T} \qquad \textit{Equation 7}$$

where N_F is the number of flocculated droplets present in the emulsion; N_{NF} is the number of non-flocculated droplets present in the emulsion; and N_T is the total number of all the droplets in the emulsion (N_F+N_{NF}). This definition does not make distinction on whether the droplets are present as monomers, dimers, trimers, tetramers etc.

The most common ways of obtaining information on emulsion flocculation in the laboratory are through microscopy and particle size analysis. Others are measurement of creaming rate and rheological parameters (Chanasattru et al., 2007).

2 Flocculation rate

The rate of flocculation is defined as the change per time in the number of fraction of droplets contained within flocs. To determine the rate of flocculation, it will be necessary to measure changes in the number of monomers, dimers, trimmers, tetramers and other multimers. Microscopy, rheology, creaming rate and particle size analysis can be used to determine flocculation rate (Chanasattru et al., 2007).

3 Floc size and shape

It is difficult to determine the size and dimensions of flocs because they are usually non-spherical and containing droplets of non-uniform sizes. Therefore, the size and shape of flocs are influenced by the number of droplets that they contain and the three-dimensional arrangement of these droplet particles within the floc (Bushell et al., 2002). When average droplet sizes are known, the number of droplets on flocs can be used to estimate floc size. The dimensions and actual sizes of flocs can be determined practically by using microscopy (Bushell et al., 2002; Zhang et al., 2010). Some limitations to the use of microscopy in determining flocs specification include alteration in floc structure during sample preparation, and limitation of only two-dimensional image of flocs, although newer microscopy techniques including the confocal laser scanning microscopy can provide three-dimensional images. Floc internal structure is another characteristic that may be relevant in specifying and describing flocculation. The arrangement of the droplets in flocs is often influenced by the nature of the droplets and formation mechanism (Bushell et al., 2002).

4 Floc strength and breakage

The ease of breakage of aggregated droplets can be used to assess the strength of flocs. Theoretically, the interaction potential, defined as the energy required to pull two aggregated droplets from their initial separation to an infinite distance apart, can be used to characterize the strength of flocs (Quemada et al., 2002). Because of the extremely small sizes of the droplets involved in flocculation, and the low magnitude of the forces involved, interaction potentials are practically difficult to determine. The use of advanced technology such as atomic force microscopy, optical tweezers, magnetic chaining techniques and micro-manipulation of flocs with pipettes has been reported to make the determination of interaction potentials possible (Dagastine et al., 2005; Mellor et al., 2005; Jarvis et al., 2005).

5 Droplet flocculation

Microscopy is widely used in characterizing flocculation because it is simple to use. Although optical microscopy is the most commonly used, others including electron and atomic force microscopy are also used depending on the properties of the emulsion or type of study. Optical microscopes are relatively inexpensive, readily available and easy to operate. It is thus widely used to characterize flocs (Loren et al., 2007). A typical procedure involves a microscope connected to a digital camera and a computer such that flocs can be observed by placing samples of the emulsion on the microscope slides. Various qualitative and quantitative parameters can be obtained from the images so obtained through the use of imaging software (Russ, 2004; Suksri and Pongjanyakul, (2008).

In non-flocculated emulsions, droplet images are evenly distributed compared to the closely packed droplet images in flocculated emulsions. Other information about the flocs that can be obtained from the microscope images includes size and morphology. Information on the nature and strength of the attractive forces responsible for flocculation can be inferred from critical observation of the microstructure. When flocs are very compact with regular shapes, the attractive forces responsible for their formation can be said to be weak, whereas strong attractive forces are responsible for flocs that are more open with irregular shape. This is because weak forces of attraction allows for rearrangement droplets in flocs after aggregation whereas in aggregation due to strong attractive forces, there is less chance for droplet rearrangement. Through this inference, bridging flocculation (strong interaction) can be distinguished from depletion flocculation (weak interaction) (McClements, 2007). Limitations to this method include the alteration of flocs characteristics during slide preparation; limitation of most microscopy to two-dimensional images; and the difficulty in identifying flocculation in concentrated emulsions (McClements, 2007).

Particle size analysis can also be employed to obtain important information on particle aggregation. This involves the use of instrumental analytical techniques, such as light scattering, electrical pulse-counting, ultrasonic spectrometry or NMR automated for particle sizing in order to measure the particle size distribution of an emulsion (Binks and Lumsdon, 2001).

A practical consideration is to ensure representative samples are used especially in emulsions with large flocs which tend to cream easily. Homogeneity of emulsions can be achieved through stirring or agitation prior to sampling. Apart from rapid creaming in emulsions with large flocs, the following are other problems with potential negative effects on particle sizing analyzers: (1) the method and conditions of sample preparation may influence the extent and nature of droplet aggregation; (2) non-homogeneity of particle sizes, which limits application in analysis and (3) the difficulty in identifying flocs as distinct from individual droplets (McClements, 2007).

It is important to carefully distinguish between coalesced droplet, flocs or droplet enlargement due to Ostwald ripening. To do this, the following methods can be applied: the particle size distribution of the emulsion is first measured; this is followed by the addition of a deflocculant which breaks down any flocs present in the emulsion; and the particle size distribution is measured again. The particle size distribution is expected to remain unchanged if there were no flocs present in the original emulsion in which case particle growth can be attributed to coalescence or Ostwald ripening. On the other hand, if the particle size distribution returns to the value of the original stable emulsion, particle growth should be attributed to flocculation (McClements, 2007). Thus, particle size analysis can be combined with microscopy to characterize flocs (Roland et al., 2003). In this research work, digital research microscope was used to monitor the growth of the emulsion droplets.

2.4.3 Coalescence

Coalescence refers to the merging of two or more droplets to form a single and larger droplet. It involves the collapse of one droplet into another. The formation of large droplets increases the propensity for rapid creaming or sedimentation, and eventual phase separation (Yarranton et al., 2007). In practice, it is important to understand the factors that make a given emulsion susceptible to coalescence. For example, the knowledge of the effects of product composition, applied mechanical forces like agitation, storage time and temperature on the susceptibility to coalescence will be important for the stability of food emulsions (de Gennes, 2001).

The process of coalescence may be homogeneous, where coalescence rate does not depend on the droplet size; the process is said to be heterogeneous when the rate of coalescence depends on the droplet size (Deminiere et al., 1998). Generally, emulsions with larger droplets coalesce faster than those with smaller droplet sizes. The tendency for coalescence is high when droplets are in contacts for a long time compared to freely moving and colliding droplets (van Aken and van Vliet, 2002).

Physicochemical basis of droplet coalescence

For coalescence to occur, the surfaces of the droplets involved must come into close contact. Coalescence may result from droplet collision or from extended contact. When the attractive forces between droplets are stronger than the repulsive forces, droplets may coalesce on collision. In concentrated emulsions, an extended close contact between droplets may lead to the disruption of the interfacial layers separating them. Thus, for coalescence to occur, either by collision or extended contacts, the interfacial barriers separating the droplets must be broken (Salager et al., 2000). The process and mechanism of coalescence are influenced by the nature of emulsifier used in the emulsion (Jafari et al., 2008). Coalescence is the ultimate form of emulsion instability that should be avoided. Other forms of instability will, with time, result in coalescence, droplet flocculation, creaming, and sedimentation; hence, excessive shearing must be avoided to enhance emulsion stability.

Experimental characterization of droplet coalescence: through microscopy

Optical microscope can be used to directly observe droplet coalescence by placing sample emulsion on a microscope slide, and observing changes in droplet size distribution as a function of time (Sakai et al., 2001). This system involves the monitoring of size and number of droplets with time. Coalescence can also be observed directly by using rapid cameras although this is seldom feasible in practice (Dickinson, 1992). Two distinct stages can be observed in the process of droplet coalescence. Firstly, there is film thinning (also known as lag phase) in which the droplets are in contact at the interface but still separated by thin films. The second phase is the disruption of the separating film (also known as the coalescence phase) leading to the merging of the droplets to form larger droplets or merging into the liquid bulk.

The major limitation in the use of microscopy to monitor droplet coalescence is that droplets with sizes less than 1 µM cannot be observed with optical microscope. The slow process of coalescence also makes it impossible to be monitored directly with the aid of optical microscope.

Experimental characterization of droplet coalescence: particle size analysis

Another method to characterize droplet coalescence is the use of automated instrumental particle sizing techniques such as light scattering, ultrasonic spectrometry, electrical pulse-counting or NMR to monitor time-dependent changes in particle size distribution (McClements, 2007). An important consideration with the use of this method is to ensure representative sample of the emulsion bulk. This can be ensured by stirring or agitation to ensure homogeneous distribution before sampling. Particle sizing techniques can give reliable results when used with emulsions that are at the initial stage of coalescence in which case the droplet sizes are relatively small. When extensive coalescence has taken place, this method is not effective to characterize coalescence (Roland et al., 2003). Since increase in droplet sizes is often used as indication for coalescence, it is important to differentiate between the different causes of increased droplet size. This is because; flocculation and Ostwald ripening can also lead to increase particle size. While it is much easy to differentiate between flocculation and coalescence, it is practically more difficult to differentiate between coalescence and Ostwald ripening (Madras and McCoy, 2003).

Experimental characterization of droplet coalescence: oiling off tests

Separate oil layers are usually formed in oil-in-water emulsions following extensive droplet coalescence. This is often referred to as ‘oiling off’. The amount of separated oil can be estimated and used to characterize the level of coalescence that has taken place. This can be done by expressing such amount relative to total oil content of the emulsion (McClements, 2007).

Experimental characterization of droplet coalescence: accelerated coalescence tests

Various methods can be used to simulate harsh environmental realities in accelerated manner in order to characterize droplet coalescence of emulsions. Such methods include mechanical agitation method and centrifugation method.

Mechanical agitation methods involve subjecting the emulsion to some forms of mechanical shaking or agitation which could be achieved through stirring, homogenization, flow through a pipe or whipping. The stability of the emulsion to coalescence is characterized by determining, through a controlled increasing shear stress, the shear stress at which coalescence is first observed. Alternatively, shear stress may be kept constant while the time it takes an emulsion to coalesce is determined (Dickinson and Williams, 1994; Puthli and Vavia, (2009). Stability to whipping-induced coalescence can be similarly determined by measuring the particle size distribution and the droplet microstructure after whipping for specified time and at given speed (van Aken, 2001; Hotrum et al., 2005). The whipping time can be varied while maintaining a constant shear stress. The time when coalescence is first observed can be used to characterize coalescence. Alternatively, a critical shear stress at which coalescence takes place can be used for characterization when shear stress is varied over a specific whipping time period.

Using specified time and speed, centrifugation can be used to characterize the rate of emulsion coalescence (Badolato et al., 2008). This can be achieved by monitoring droplet size distribution before and after centrifugation. Parameters that can be used in such determination are the minimum centrifugation force or the length of time an emulsion can tolerate before coalescence is observed (van Aken et al., 2002).

Limitations of accelerated coalescence tests

There are few important limitations to the interpretation and application of accelerated coalescence studies. Although, indications of long-term stability are obtainable over a relatively short time, the simulated environmental factors through the use of whipping, centrifugation, shearing or homogenization are not exact representation of environmental stresses encountered by emulsions during storage (McClements, 2007). For instance, the arbitrary force limits required to pass accelerated coalescence tests may be way too high compared to the forces encountered during storage. In addition to this, other factors including biochemical changes (like lipid oxidation, protein hydrolysis) that may result in coalescence are not examinable through conventional accelerated coalescence tests. Thus, results from accelerated tests should be interpreted with caution.

Partial coalescence

Partial coalescence results from the formation of irregularly shaped aggregate when two or more partly crystalline lipid droplets within an oil-in-water emulsion come into close contact. The aggregates are formed in such a way that the mechanical strength of the fat network within the droplets prevents total coalescence. This allows the droplets to retain their original shapes (Walstra, 2003; Fredrick et al., 2010).

Partial coalescence is important especially in emulsions containing animal fats and other products like milk and other dairy products where crystalline globules are formed over a range of temperature (Walstra, 2003). The crystalline droplets come in contact during collision in the aqueous continuous phase or after adsorption to the surfaces of air bubbles that are formed during shearing. Thus, partial coalescence can result from the application of shear forces and/or temperature cycling to ice-cream, whipped toppings, butter and margarine which contain partly crystalline milk fat globules leading to increased viscosity and gravitational separation. Droplet aggregation due to extensive partial droplet coalescence will eventually lead to phase inversion in butter and margarine. On the other hand, aggregated fat droplets in ice-cream and whipped cream will form networks which surround the air molecules and extend through the aqueous bulk providing much mechanical strength that stabilizes the system (Fredrick et al., 2010).

Physicochemical basis of partial coalescence

Partial coalescence takes place when solid fat crystals from one droplet penetrate into the liquid oil portion of another droplet (Fredrick et al., 2010; Walstra, 2003). Fat crystals in oil-in-water emulsions are usually surrounded by the aqueous continuous phase. When such fat crystals penetrate into another droplet however, the new surrounding environment is the oil. This new environment reduces the interfacial energy and is more thermodynamically favorable towards droplet aggregation.

Since the penetration of solid fat crystals into the liquid oil phase is a major requirement for partial coalescence, partial coalescence occurs only in emulsions that contain partially crystalline regions. Droplets in complete liquid forms will undergo the normal coalescence and if in complete solid form, will undergo flocculation (Blanchette and Bigioni, 2006).

Factors that affect the rate and nature of partial coalescence include the morphology, concentration, location and dimension of the fat crystals within the droplets; the amount of shear stresses the emulsion is exposed to; the chemical composition, cooling rate, temperature, droplet size distribution, emulsifier type and the presence of any impurities in the emulsion system; the nature of the attractive and repulsive interactions between the droplets (Walstra, 2003).

Characterization of partial coalescence

Partial coalescence is characterized by three major elements: the properties of the fat crystal, the microstructure and macroscopic properties of the emulsion. All of which are important factors that predispose emulsions to partial coalescence.

Fat Crystal Properties

The properties of the fat crystals in an emulsion morphology, concentration and location are an important determinant of the susceptibility of an emulsion to partial coalescence. The application of experimental procedures that are based on the differences in the physicochemical properties of the solid and liquid phases is one of the ways to characterize fat crystal properties. Such experimental characterization includes the determination of crystal compressibility, density, molecular mobility, birefringence, and/or packing. Most importantly, the final melting point of the fat crystal; variation of solid fat content with temperature; the morphology and location of the crystals within the droplets; the crystal arrangement of the fat molecules; and the influence of droplet crystallization on the overall stability of the emulsion are important considerations. Analytical measurement for these purposes includes differential scanning calorimetry, nuclear magnetic resonance, ultrasonic velocity measurement; density measurements and electron spin resonance (Coupland et al., 2002; Rousseau, 2000).

Microscopy (optical, electron or atomic force) can be used to study the location of fat crystals (Rousseau, 2006). Adsorption and radiation techniques can be used to measure the packing and arrangement of the molecules in the crystals (Rousseau and Hodge, 2005; Tang and Marangoni, 2006). The spacing between the molecules in the fat crystals can be determined by utilizing X-ray diffraction techniques (Hartel, 2001).

Emulsion Microstructure

Optical and electron microscope can be used to monitor the temperature-dependent changes that occur in fat crystals that lead to formation of extensive network of aggregate and partial coalescence (Relkin and Sourdet, 2005). If a partially coalesced emulsion is heated to certain temperature such that the fat crystals melt, complete coalescence will occur due to the merging of the oil droplets. This will lead to increased droplet sizes, oiling off and phase separation. Particle sizing analysis and the measurement of oiling off, as discussed earlier can be used to characterize the warm emulsion at this stage.

Macroscopic Properties

Macroscopic properties can be easily observed in partially coalesced emulsions especially the formation of large aggregates and the increasing viscosity. These macroscopic changes can be monitored by direct measurement, for example, of viscosity (Dalgleish, 2006).

2.4.4 Ostwald Ripening

Ostwald ripening describes the growth of larger droplets at the expense of smaller ones. It occurs as a result of mass movement of dispersed phase from one droplet to another leading to the growth of large droplets at the expense of others (Kabalnov, 2001; Taylor, 2003). Its effect is often insignificant in most emulsions because of the poor water solubility of oily additives in the emulsion. However, in instance where more water-soluble flavors and essential oils are present in oil-in-water emulsions, the effects of Ostwald ripening on the total stability of the emulsion could be very significant. Ostwald ripening essentially involves the movement of soluble materials or particles from smaller droplets to larger ones. Thus, the larger the receiving droplets, the more soluble the moving particles become. This process leads to the shrinking of the smaller droplets and growth of the larger ones invariably increasing the mean droplet size of the emulsion with time (Meinders and van Vliet, 2004).

Measurement of Ostwald ripening

The methods for monitoring droplet coalescence, as discussed earlier, can be used to monitor Ostwald ripening. Techniques that monitor particle size distribution with time, such as microscopy and particle sizing analysis are applicable in characterizing Ostwald ripening (Sadtler et al., 2002; Hoang et al., 2004). By measuring the change in particle size distribution with time (time dependence and linearity) and/or considering the predisposing factors influencing droplet size growth, a distinction can be made between coalescence and Ostwald ripening. Coalescence depends largely on the type of emulsifier used in the emulsion whereas; Ostwald ripening is fairly independent of the emulsifier type (Sadtler et al., 2002).

2.4.5 Phase Inversion

Phase inversion is the process whereby an oil-in-water emulsion changes to a water-in-oil emulsion, or vice versa (Kralchevsky et al., 2005). Apart from being a form of instability, phase inversion is an essential step in the manufacture of some emulsion products including butter and margarine, where an oil-in-water emulsion is converted into a water-in-oil emulsion (Bouchama et al., 2003; Walstra, 2003). Apart from this instance, phase inversion is generally undesirable in prepared emulsions.

Factors that trigger phase inversion in prepared emulsions include disperse phase volume fraction, nature and concentration of emulsifier used, solvent type, the presence of other additives, storage temperature or mechanical agitation (Bouchama et al., 2003; McClements, 2005). It is more common for emulsions to separate into their component phases than undergo phase inversion. The physicochemical basis of phase inversion is very complex, and it often involves aspects of droplet flocculation, coalescence, and disruption (McClements, 2005). Phase inversion can be either surfactant induced or fat crystallization induced. Surfactants can induce phase inversion if there are changes in their molecular geometry arising from environmental changes like addition of salt, alcohol, or another emulsifier to the emulsion, or drastic temperature changes (McClements, 2007). Fat crystallization-induced phase inversion results from changes such as temperature, shearing and alteration of interfacial layer thickness, in the emulsion that favors extensive partial coalescence (Walstra, 2003).

Measurement of phase inversion

Various analytical determinations can be used to characterize phase inversion. These include electrical conductivity, optical properties, microscopy, viscosity, and droplet size analysis. The electrical conductivity of an emulsion depends on the emulsion type. Since an oil-in-water emulsion has an aqueous continuous phase, a high electrical conductivity is expected. On the other hand, a water-in-oil emulsion has oily continuous phase which conduct electricity poorly. Thus, a drastic change in the electrical conductivity of an emulsion may indicate phase inversion (Allouche et al., 2004; Tyrode et al., 2005).

Generally, the viscosity of an emulsion is a reflection of the viscosity of the continuous phase. There is usually a significant change in the viscosity of an emulsion when phase inversion occurs with the new viscosity reflecting the new continuous phase. Thus, phase inversion can be monitored through the measurement of emulsion viscosity used to follow phase inversion in emulsions (Allouche et al., 2004; Tyrode et al., 2005).

The optical properties of an emulsion depend on the nature of the droplets, droplet concentration and size. These properties will change when phase inversion occurs. The use of refractory colorimeter or spectrophotometer can be used to measure these optical properties (Binks and Rodrigues, 2003).

Microscopy provides information on the nature and characteristics of the structural changes occurring to the emulsion droplets during phase inversion. This can be done, for example, through the use of a dye that is soluble in only one of the phases. If oil-soluble dye is used for an oil-in-water emulsion, initial microscopy reveals colored droplets on colorless background. After phase inversion of such emulsion, microscopy reveals colorless droplets on a colored background (Rondon-Gonzalez et al., 2006).

Phase inversion is associated with significant changes in emulsion droplet size. Therefore, the measurement of the droplet size distribution can be used to monitor phase inversion.

2.5 Testing Emulsifier Effectiveness

Oil-water mixtures are thermodynamically unstable, emulsifiers are important components of emulsions. The overall quality and long-term stability of emulsions depends largely on the type of emulsifiers used. The ability of emulsions to withstand various environmental conditions during storage is a function of emulsifier effectiveness.

Various natural and synthetic materials have been used to emulsify emulsions. Various factors to be considered for determining the most appropriate emulsifier for particular emulsions are physical and chemical compatibility, minimum emulsifier concentration for emulsion stability, the ability of the emulsifier to produce droplets with acceptably small sizes during homogenization and ability to prevent droplet aggregation. Other determinant factors of emulsifier choice are properties of the emulsion to be prepared. These include the oil type, ionic strength, ion type, pH, ingredient interactions, and thermo-mechanical history (Calvo et al., 2008; McClements, 2007).

Due to these reasons, suitability of emulsifiers is difficult from their chemical structure or known physicochemical properties. It is therefore necessary to investigate the suitability of emulsifiers under the conditions expected of the emulsions to be prepared. The two most common way of testing emulsifier efficiency are emulsifying capacity and emulsifying stability index (Kato et al., 2006). This can be in addition to testing prepared emulsions for stability to a range of environmental stresses.

2.5.1 Emulsifying capacity

Emulsifying capacity (EC) refers to the minimum quantity of emulsifier required to stabilize an emulsion. Emulsifying capacity of a water-soluble emulsifier is the maximum quantity of oil that can be dispersed in and aqueous continuous phase containing a specific amount of the emulsifier without phase separation or inversion of the emulsion (Webb et al., 2006). This can be determined experimentally, for example by titrating oil in an aqueous emulsifier solution in a continuously agitation vessel using a high speed mixer. The EC is determined as the point where the emulsion breaks down or inverts, which can be determined through optical, rheological or electrical conductivity measurements (Kanterewicz et al., 2006). The higher the oil volume added to the emulsion before it breaks, the higher the emulsifying capacity of the emulsifier.

There are a number of limitations to this method. The type of blender, the speed of mixing, droplet size, the measurement temperature, initial emulsifier concentration and the rate of oil titration will affect the end point. Under similar conditions however, the method may be effective in determining comparative emulsifying capacity of a number of emulsifiers and for measuring comparative efficiencies of different emulsifiers (Webb et al., 2006).

2.5.2 Emulsion stability index and emulsifying activity index (EAI)

Emulsifying stability index ***(ESI)*** is the measure of the ability of an emulsifier to prevent increases in droplet sizes through aggregation, coalescence or ripening (McClements, 2007). It is determined by measuring the change in emulsion droplet size after storage for specified length of time and/or after exposure to some environmental stress like temperature variation, controlled agitation or stirring etc. Little or no increase in the particle sizes indicates better stability index of the emulsifier (Molina et al., 2001). As a parameter, ESI can be defined as the time required for the mean particle diameter to double in size. In the laboratory, values for EAI and ESI can be determined using turbidimetry method (Mu et al., 2011). Prepared emulsion is pipetted from the bottom of the container into 0.1% sodium dodecyl sulfate (SDS) immediately (time = 0) and after predetermined time of homogenization. The absorbance of the SDS solutions is determined at appropriate wavelength. Absorbance at time zero expresses the EAI while the ESI is calculated from the equation:

$$\mathrm{ESI} = \frac{T_0 \times t}{T'} \qquad \text{Equation 8}$$

Where T_0 = the turbidity at 0 minute after homogenization, T'= the change in turbidity between 0 and predetermined time and t = interval time between 0 and the predetermined time.

The major limitation of ESI as a measure of emulsifier efficiency is the lack of consideration for the mechanism of the increase in particle size. It should also be noted that particle size increase is not always linear which makes the time-dependent measurement of ESI problematic. The rate of particle size increase usually depends on the initial droplet size, concentration and continuous phase rheology. These factors may vary from emulsion to emulsion and will need standardization for use in different systems. Limitation also arises from the short-time measurement as particle growth naturally occurs at a relatively slow rate. The long time effect of the emulsifier may thus be difficult to predict from the time-dependent ESI. The use of ESI is effective when comparing different emulsifiers (Mu et al., 2011).

2.5.3 Environmental stress tests

Prepared emulsions are often exposed to various environmental conditions to assess the effectiveness of the emulsifier. Such conditions to be varied include pH, ionic strength, solution composition, temperature and mechanical forces. The ability of emulsifier to keep an emulsion stable during extended storage, in the presence of mechanical forces, freeze-thaw cycling, thermal processing, dehydration and varied ionic/pH environment, can provide the indication of the emulsifying effectiveness of the emulsifier (Gu et al., 2005).

The effects of storage on emulsion can be investigated by keeping the emulsion in a temperature-controlled and light-controlled environment for a fixed period of time. The emulsion characteristics can then be assessed and compared to the initial properties. Storage conditions and duration to be used in the test should normally reflect the expected shelf-life and storage conditions of the product (Chinwanitcharoen et al., 2004).

Emulsion products are transported like other goods. During their shelf-life, they are subjected to mechanical forces. Stability to these conditions can be assessed by subjecting sample emulsions to a well-defined shear force for a specified period of time. This may include the use of emulsion-containing sealed conical flasks swirled at a fixed speed in a temperature controlled shaker or emulsions subjected to high speed blending and shearing at a fixed speed for a certain time (McClements, 2007).

Emulsion products such as frozen sauces, dips or deserts may undergo freezing and thawing during storage and use. It is thus important for such emulsions to be stable to cycles of freezing and thawing. To assess stability of emulsions to freezing and thawing, sample emulsions can be placed in freezer at a fixed temperature and for specified period of time and then thawed at room temperature. The samples are then tested for stability. The process can be repeated a number of times (Thanasukarn et al., 2006).

The pH and mineral composition of emulsions vary widely. It is important to assess the influence of varying pH and mineral composition on the stability of emulsions. This can be done by preparing emulsions with a range of pH values and mineral compositions and then store for fixed period of time. The properties of such emulsions are then tested to assess stability. Typically, prepared emulsions are acidified and/or basified employing 0.1M HCL or NaOH to pH 2-10. The influence of the changes in the pH is determined employing Turbiscan® and through physical observation of creaming/sedimentation (Laplante et al., 2005; Qi et al., 1997). In the current study, the emulsions were subjected to varying pH, salt, homogenization speed, vinegar addition.

The effect of such additive/condition was studied over a storage duration using Tubiscan® MA 2000 for droplet migration monitoring and the microscope for droplet size growth.

2.6 Bambara Groundnut

2.6.1 General overview of Bambara groundnut

In southern Africa especially South Africa, Botswana and Zimbabwe, Bambara groundnut [BGN] (Figure 2.8) is an important agricultural product for subsistence and commercial purposes. Its popularity among African farmers has grown due to its suitable agricultural properties including ease of cultivation; adaptability to wide range of weather conditions like drought and heavy rainfall, hot and cold temperature; minimal soil-type and cultivation requirements; and relative short period (4-5 months) of maturation. It thrives more easily than other leguminous plants with resistance to high temperature (Alakali et al., 2010).

As a traditional food crop in Africa, it is eaten fresh or boiled after drying due to its high nutritional value. The flour from ground seed is used to make cake for consumption. BGN is a good and cheaper source of nutritional protein compared to animal and other plant sources (Azam-Ali et al., 2001; Kaptso et al., 2008; Alakali et al., 2010). According to Rachie and Roberts (1974), BGN is the most important grain legume after groundnut (*Arachis hypogea*) and cowpea (*Vigna unguculata*).

2.6.2 Composition of Bambara groundnut

In a study to analyze its nutritional composition, Doku (1996) reported relatively high lysine content while another study by Amarteifio et al. (1997) showed a breakdown of 53.1% carbohydrate, 17.4% protein, 6.1% fat, 6.1% fiber, and 3.4% ash, as well as low levels of calcium, iron, sodium and potassium. The protein in BGN is said to be richer as it has been reported to contain more essential amino acid (methionine) than any other legume (FAO, 1970; Aremu et al., 2006). In addition to the relatively high protein content in BGN, the total nutritional composition reflects an excellent balance unusual in single plant products. Thus, it has the potential to combat under-nutrition in rural areas (Mbata, 2009).

2.6.3 Dietary and medicinal uses of Bambara groundnut

Medicinally, apart from enhancing dietary balance and the potential to prevent nutritional deficiencies especially kwashiorkor, the consumption of BGN has claimed to aid digestion (Baryeh, 2001; Lawal et al., 2007; Mpotokwane et al., 2008). Ethnobotanical studies have shown its medicinal use in Africa for a variety of diseases. In Burkina Faso, BGN is used

Figure 2.8 Black eyed Bambara groundnut seeds

to manage gastrointestinal disorders, body wasting and skin problems (Nacoulma-Ouedraogo, 1996). The use of the seeds for medicinal purposes to treat diarrhea in South-East Nigeria and Kenya has also been reported (Atiku, 2000). Although the scientific basis for its medicinal uses is yet to be established, the roasted bean of BGN is consumed to treat polymenorrhea, water suspension of the flour is used to resort hematomas, its high soluble fiber content is believed to be beneficial in the prevention of colon cancer and heart disease, the immature seeds are chewed in their fresh forms to control nausea and vomiting especially in pregnancy, the water of boiled seeds is used as hemostatic drink and in the management of various malignancies (Goli et al., 1991; Hye-Kyung et al., 2004; Akindahunsi and Salawu, 2005).

2.6.4 Research studies on Bambara groundnut

Very limited studies have been conducted on BGN and such studies are concentrated on cultivation and agricultural yield (Adeniji et al., 2008). As a result of the potentials presented by BGN in food security and industrial raw materials source (starch and protein), there is growing research interest in the application of its products in food industry. For example, Baryeh (2000) investigated the physical properties of West Africa-sourced BGN while a similar study on Southern Africa-sourced BGN was conducted by Mpotokwane et al., (2008). In both cases, BGN was reported to possess physical properties that will allow commercial and industrial uses.

2.6.5 Emulsification capacity of other legumes and their products – literature evidence

Legume seeds are very rich in proteins and serve as rich sources of dietary fiber and carbohydrates (Messina, 1999). Products of legumes have been reported in various studies to possess emulsifying property.

Chau and Cheung (1998) investigated the functional properties of the flour of three popular legumes widely cultivated in Egypt, India and other Asian countries - *Phaseolus angularis*, *Phaseolus calcaratus* and *Dolichos lablab*. The flour of all three legumes showed satisfactory foam capacities, water- and oil-holding capacities, as well as gelations capability comparable to soy bean. The flour of all three legumes showed pH-dependent emulsifying activities with emulsion stabilities greater than 80% from pH 2 to 10 except for pH 4. The authors also assessed the protein concentrates from these legumes for similar functional properties. The concentrates from all three legumes demonstrated emulsion stability above 93% over pH range of 2 to 10 (Chau and Cheung, 1997).

Similarly, Chel-Guerrero and co-workers reported significant emulsifying activities (46.78−53.84%) of the flours and protein isolates from *Phaseolus lunatus* and *Canavalia ensiformis* for a pH range of 6 to 10. Up to 100% emulsion stability was reported for the flour and protein isolates from *C. ensiformis* at pH 7 and 8 (Chel-Guerrero et al., 2002).

European legumes such as lupin, pea and broad bean have been shown to possess emulsifying property. According to Makri and others, protein isolates from lupin, pea and broad bean showed similar but high emulsion-stabilizing ability. The emulsions were however destabilized by the addition of sodium chloride (Makri et al., 2005). In another study, lupin (*Lupinus mutabilis*) demonstrated a concentration- and pH-dependent emulsifying property (Sathe et al., 1982).

The flour of cowpea (Vigna unguiculata) was reported to very good emulsifying capacity that is enhanced by the addition of up to 0.4% sodium chloride (Abbey and Ibeh, 1988). Sodium chloride, up to 0.4M also enhanced the emulsification capacity of the flour of winged bean (*Psophocarpus tetragonolobus*) (Narayana et al., 1982).

Ramanatham and co-workers investigated the emulsifying capacity of groundnut protein and reported eptimal emulsification at 5 mg/ml protein concentration. The emulsifying capacity of groundnut protein was highest pH 10 and the addition of low concentrations of sodium chloride enhanced it (Ramanatham et al., 1978). Other legumes whose flour and/or protein isolates have shown significant emulsifying capacity include chickpea (*Cicer arietinum*) (Kaur and Singh, 2005), popular indian legumes *Canavalia cathartica* and *Canavalia maritime* (Seena and Sridhar, 2005*)*, beach pea (*Lathyrus maritimus*) (Chavan et al., 2001), peanut (Yu et al., 2007), field pea and pecan (McWatters and Cherry, 1977).

Thus, legumes have generally shown the potential for emulsification. No study has evaluated the emulsifying capacity of BGN. As a popular legume in Africa, the potential for industrial use of BGN in food is high. It is therefore important to assess the ability of BGN to stabilize emulsions.

2.6.6 Possible emulsifying properties of Bambara groundnut

A number of food, pharmaceutical and industrial products are prepared as emulsions (Patravale and Mandawgade, 2008). Bambara bean is a consumable legume. Its flour will be a good emulsifier if found suitable with the added advantage of its nutritional properties. It is therefore, a worthwhile study to investigate the emulsifying properties of Bambara groundnut flour.

Various synthetic and natural polymers have been used as emulsifying agents to improve the stability of food, pharmaceutical and cosmetic emulsions (Mirhosseini et al., 2008). These include acacia gum, agar, egg yolk, gelatin, casein, guar gum, tragacanth, alginic acid, karraya gum, carrageenan, carbomer resins, cellulose ethers, carboxymethylchittins, polyethylene glycol, stearyl alcohol, cetyl alcohol, cholesterol, bentonite, magnesium hydroxide, aluminium hydroxide, magnesium aluminium silicate, kaolin, various starch, among others. The choice of emulsifying agent is often dependent on the type of emulsion and the physicochemical properties of other emulsion components. Studies have shown that natural products (proteins, carbohydrates) possess excellent emulsifying properties (White et al., 2008). The search for emulsifiers among natural plant/food products is particularly desirable due to the non-toxic nature of the products and their already established food and medicinal uses. In addition, plant products are more affordable and readily available leading to an increasing trend in the use of plant-derived emulsifiers for food and pharmaceutical applications (Wang et al., 2010a, 2010b).

2.7 Conclusion

Emulsions are popular as food, pharmaceutical and industrial formulations. For stable emulsions, a suitable emulsifier is required. The nature and type of such emulsifier determines physicochemical properties of the emulsions. Various natural and synthetic compounds have been used to stabilize emulsions. The emerging trend is the search and use of plant-derived (green) emulsifiers. Such products may serve multiple purposes including nutritional and thickening purposes. BGN is a popular leguminous plant with long history of nutritional use having high protein and carbohydrate contents few article have reported the starch (Adebowale et al. 2002). However no attempt has been made to stabilize oil-water using BNGF/S, in this work it is of interest to study the stability of oil-water emulsion using the laboratory techniques like Tubiscan® for destabilization kinetics and digital microscope for change in droplet size.

CHAPTER THREE

Research Methodology

3.1 Study Design

This chapter describes the design and methodology used for the research project. It describes the source of materials, sample preparation, sampling, stability test procedures, methods of analysis and interpretation of results.

The research was laboratory-based, and was divided into two phases. The first phase involved determination of the optimal oil/water/BGNF/S composition for BGNF/S-emulsified emulsions. The most stable emulsions from this first phase (one BGNF-stabilized, and one BGNS-stabilized) were subjected to a second phase study. The second phase study involved the subjecting of the emulsions to simulated physicochemical and mechanical conditions in order to observe the ability of the emulsions to withstand such conditions.

3.2 Materials

Bambara groundnut was obtained from Triotrade, Gauteng CC., South Africa. Sunflower oil was purchased from local supermarket in Cape Town. All other materials and equipment were obtained from the Departments of Chemical Engineering and Food Technology laboratories of the Cape Peninsula University of Technology. Chemical reagents used were of analytical grade. The major equipment used in this study were Turbiscan® (Turbiscan® MA 2000, Formulaction, Toulouse), homogenizer (Ultra-Turrax T25, Janke and Kunkel, Staufen, Germany) and a digital research microscope (Ken-a-vision, Kansa city, MO, USA).

3.3 Methodology

3.3.1 Preparation of Bambara groundnut flour

Bambara groundnut was milled using a hammer mill (Trapp TRF 400, Animal ration shredder/hammer mill foliage, Jaraqua do sul-sc, Brasil) to produce the Bambara groundnut flour (BGNF). The flour was sieved by passing through 250 μ mesh for homogeneity (Uvere et al., 1999).

3.3.2 Isolation of starch from BGN flour

The method of Adebowale *et al.* (2002) was employed with modifications to isolate starch from the BGN flour. A typical extraction procedure involved the mixing of weighed BGN flour with water (1:10 w/v) at room temperature for 1 h. Thereafter, the mixture was allowed to stand for 5 h followed by centrifugation (3,500 × *g*, 30 min). The supernatant was discarded and the residue was subjected to similar procedure, first with water containing 2% w/v NaCl (10 min mixing and 12 h standing); and then 0.03M NaOH (10 min mixing and 12 h standing). The resultant residue was re-constituted in water and passed through a 75 µm sieve to remove the fiber. The sieved mixture which contained the pure starch was then left for 2 h to sediment after which the supernatant was decanted. The residue was air-dried (at room temperature) to yield the BGN starch (BGNS). A schematic description of the extraction procedure is shown in Figure 3.1.

3.3.3 Determination of oil:water:BGN flour/starch ratio in emulsions

The D-Optimal response surface methodology was used to generate a range of emulsions using BGNF/S and oil as variables (Taha et al., 2005) (Table 3.1). A total number of 12 emulsions templates were thus generated with duplicates. Following this design, the emulsion was prepared by gelatinizing the BGNF or BGNS (quantities as designed in Table 3.1) in sufficient water to make a 100 g mixture. Gelatinization was achieved by heating and stirring the BGNF/S-water mixture on a heater equipped with magnetic stirrer for 10 min (Cho and Lee, 2002). The resultant gel was then left to cool to room temperature before the oil was added. The addition of oil was immediately followed by homogenization using high-speed homogenizer (IKA Ultra-Turrax$^{®}$ T25, Digital Janke and Kunkel, Staufen, Germany) set at 15000 rpm for 10 min (Yusoff and Murray, 2011). The quality of the emulsions were accessed using migration rate and droplet size (Hollingsworth and Johns, 2006). After the preparation and initial stability testing of the emulsions based on the generated ratios, the most stable emulsions were identified based on creaming stability results as measured using droplet migration rates. Figure 3.2 showed the digital photograph of the homogenizer used for this research work.

3.3.4 Migration/creaming rate determination

Stability studies of the emulsions involved the use of Turbiscan® MA 2000 (Figure 3.3) and optical microscope. The two major destabilisation phenomena affecting the homogeneity of emulsions are droplet migration (creaming, sedimentation) and particle size variation or aggregation (coalescence, flocculation) (Mengual et al., 1999) but the creaming was used to determine the stability of the emulsion prepared in this research work because it was the most common destabilisation observed with Turbiscan® MA 2000 in all the sample prepared.

The assessment of the physicochemical stability of the emulsions was performed using the Turbiscan® MA 2000. Emulsion sample (7 ml) was put in the tubes (Turbiscan® cell) and the ∆BS% was recorded every 1 min over 20 min, repeated daily for 5 days.

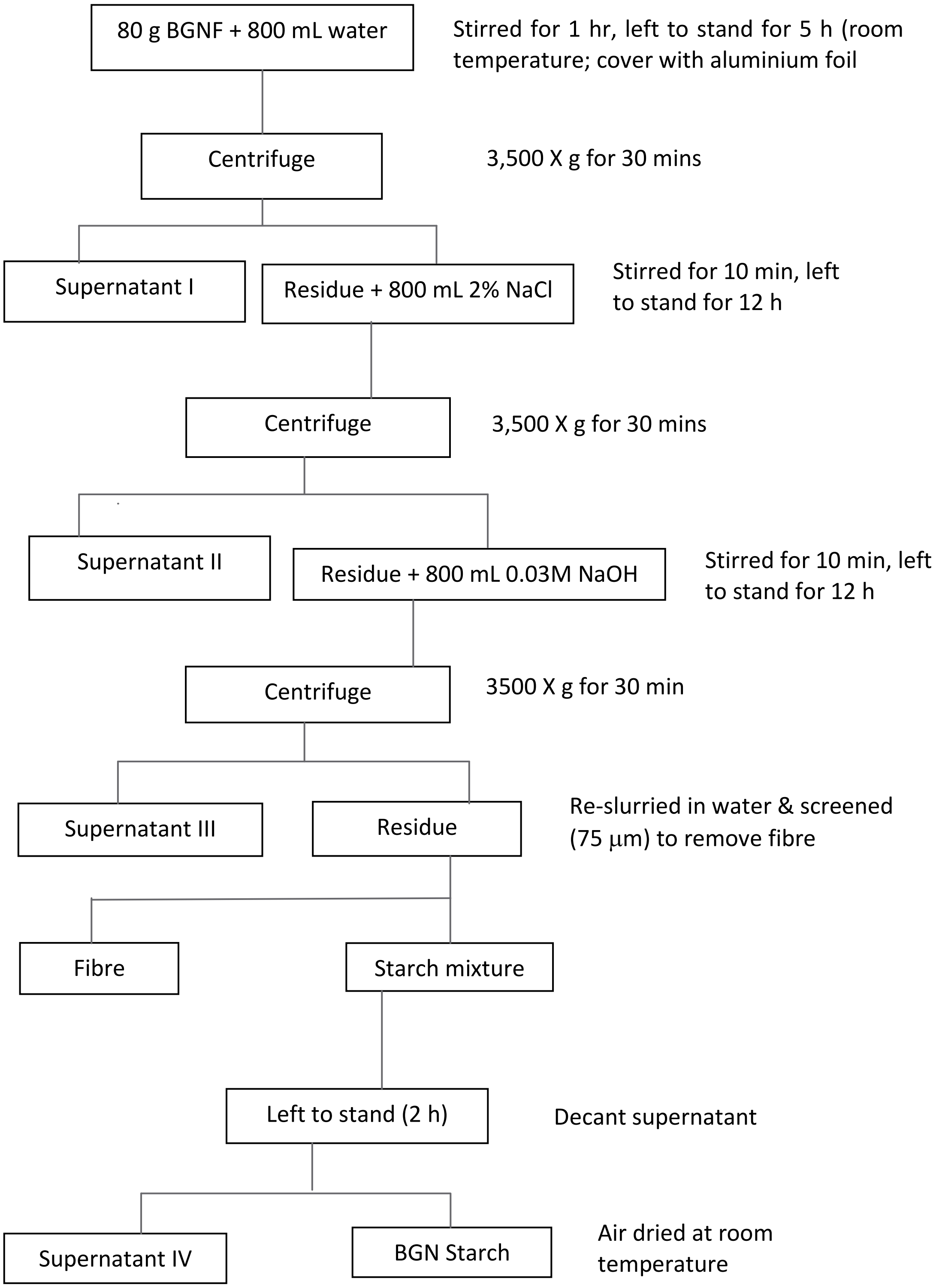


Figure 3.1 Schematic descriptions showing the process of starch isolation from Bambara groundnut flour

Table 3.1 Process variables used for the D-optimal Response Surface design for BGNF/S emulsion composition [a,b]

		Coded values		
Variable	Symbol	-1	0	+1
Flour (g)	X_1	2	6	10
Oil (g)	X_2	5	30	55

[a] Transformation of coded variable (x_i) to uncoded variable (X_i) levels could be obtained from $X_1 = 4x_1 + 6$; $X_2 = 25x_2 + 30$,

[b] This table was the same for BGNS except that the BGNS concentrations were halves of the corresponding BGNF.

Figure 3.2 Ultra-Turrax® T25 homogenizer

Figure 3.3 Turbiscan® MA 2000

Observations for creaming and sedimentation were made and the data were computed using the migration software equipped in the Turbiscan MA 2000 (Mengual et al., 1999).

The migration rate was computed by following the migration front using the migration software, available on the Turbiscan® MA 2000. Typically, the slope of the cream peak thickness kinetics was first identified. The linear portion of this slope was zoomed and copied into the migration software from where the migration rate (equivalent to the creaming index) is computed (Figure 2.6). The migration rate of creamed emulsion was used as the negative control. Percentage instability was calculated relative to the control (Hunter et al., 2011).

3.3.5 Determination of optimal BGN flour/starch for optimal emulsion.

The result was fitted to quadratic response model and analysis of variance was used to establish the effect of flour/starch and oil on the emulsion migration rate (Yuan et al., 2008). The optimum flour/starch emulsion was determined using the numerical optimisation algorithm in Design Expert software (Holm et al., 2006). The goal of the optimisation was to minimise migration rate within the range of flour/starch and oil in the emulsion (Hollingsworth and Johns, 2006).

Droplet size determination

Changes in droplet size and concentration were monitored using micrographs obtained with the aid of the Ken-a-vision digital research microscope (Figure 3.4). Micrographs were obtained by placing a drop of the emulsion on a microscope slide. The micrographs were then displayed on the computer screen through the attached Applied Vision^{TM} software. The mean diameter of representative droplets (500 per micrograph) was then obtained by using the measurement calibration in the Applied Vision^{TM} Software (Balinov et al., 1994; Traynor et al., 2013).

3.3.6 Stability of the BGN-stabilized emulsions to changes in physicochemical environment

Effects of stirring on the optimized emulsion

In order to assess the influence of stirring on the emulsion stability, the optimized emulsions 9% w/v BGNF and 5% w/v BGNS were prepared through varied homogenizer speed. The homogenization speed (rpm) used are 9000, 12000, 15000, 18000 and 21000. Samples were subjected to stability studies as described in previous sections (section 3.3.4 and 3.3.5). Relying on the theoretical framework on the ability of the highest stirring to produce the best dispersal initially, the immediate stability (just after homogenization) of the emulsion prepared at 21000 rpm (the highest practically available

Figure 3.4 Digital research microscope Ken-A-vision

speed in the current study setting) was taken to be 100%. The immediate and subsequent stability of the other emulsions were measured relative thereto (Tolosa et al., 2006).

Effects of salt (NaCl) on the stability of the emulsion

In the current study, optimal emulsion as determined earlier (section 3.3.3) stabilized separately by BGN flour and BGN starch were prepared incorporating sodium chloride (2% to 10%). The NaCl (2%, 4%, 6%, 8% and 10% w/v) was dissolved in water (continuous phase) preceding the addition of flour/starch. This was followed by gelatinization with continuous stirring on a heater and the gel formed was allowed to cool to the room temperature. The emulsion preparation generally followed the same procedure as highlighted in (section 3.3.3), except for the addition of salt to the continuous phase (water) (Fortuny et al., 2007). Samples of the prepared emulsions were subjected to stability study using visual observation over days, daily scans with Turbiscan®, and droplet size monitoring using the microscope as detailed in sections 3.3.5.

Effects of pH on the stability of the emulsion

The effect of pH on the stability of the BGN-stabilized emulsion was carried out by altering the pH of the water (continuous phase) using 0.5M NaOH or HCl. The pH of the water was measured using a pH meter, and adjusted appropriately to 2, 4, 6, 8 and 10 before the addition of flour/starch. The emulsion preparation was then completed and samples analysed for stability over 5 days (see section 3.3.4 and 3.3.5) and the stability of the emulsions were determined relative to the stability of the one prepared at pH 7 (Fortuny et al., 2007).

Effects of vinegar on the stability of the emulsion

Vinegar is a common additive to food products. It consists of acetic acid (produced by the fermentation of ethanol by bacteria) and water. It is used as flavorant in cooking, as condiment in cooked food products, in saucing and salad dressings, as mild acidic agent to acidify food products etc. it is desirable in this study to assess the influence of vinegar on the stability of food emulsion. This is because such food emulsions stabilized by BGNF and BGNS may require the addition of vinegar for some purpose. Similar to the effects of salt on the stability of the emulsion, the effect of vinegar was assessed. At 2%, 4%, 6%, 8% and 10% v/v, vinegar was dissolved in water (continuous phase) used to prepare the emulsion (Fortuny et al., 2007). Samples of the prepared emulsions were subjected to stability by visual observation over days, daily scans with Turbiscan®, and droplet size monitoring using the microscope as detailed in section 3.3.5.

3.4 Data Analysis

Analysis of variance (ANOVA) was used to establish mean differences between treatments at 5% probability (Martini and Tippetts, 2008). Duncan multiple range test was used to separate means where significant difference existed (IBM SPSS, 2012).

CHAPTER FOUR

Results and Discussion

4.1 Introduction

This chapter summarizes the findings of the investigation into the emulsifying potential of Bambara groundnut flour (BGNF) and starch (BGNS). The results are presented in two parts. The first part highlights the outcome of the optimization process which assessed various oil-water-emulsifier composition in order to arrive at an optimal stabilized emulsions (one each stabilized by BGNF and BGNS). The second part presents the influence of pH, homogenization speed, salinity and vinegar on the optimal emulsions. The discussion situates these findings within the literature.

4.2 Relationship between migration rates, droplet sizes and emulsion stability

In the current study, emulsion stability was determined by the migration rate of their oil droplet as well as the growth of the droplet size with time. The two major destabilisation phenomena affecting the homogeneity of emulsions are droplet migration (creaming, sedimentation) and particle size variation or aggregation (coalescence, flocculation) (Mengual et al., 1999). The most common destabilization process is creaming. The kinetic process of creaming starts when emulsion droplets starts migrating towards themselves and aggregate, leading to the breaking down of the inter-particulate walls and coalescence. Two stages that can be used in quantifying the rate of destabilization are the rate of droplet migration and the rate of coalescence. The higher the migration rate, the higher the creaming of the emulsion. The rate of coalescence is directly related to the increase in the droplet sizes. Thus, by monitoring the increase in droplet sizes, the rate of emulsion coalescence is determined. Generally therefore, the less the migration rate of emulsion droplets, the more stable the emulsion. Similarly, the lower the rate of increase in the droplet sizes of an emulsion, the more stable the emulsion is (McClements, 2005).

4.3 Bambara Groundnut Starch Yield

The process of starch extraction was successful, yielding 31.4% starch. This is lower than the yield (37.5%) reported by Adebowale *et al.* (2002) and the varieties used might have been different. The starch yield from the current extraction represents the starch after drying. The starch obtained was powdery, whitish, tasteless and odourless (Figure 4.1). Earlier studies have reported varied carbohydrate composition of varieties of BGN. Sirivongpaisal (2008) reported 11.4% protein and 53.1% carbohydrate in BGN while

Eltayeb et al. (2011) reported 17.7% protein and 86% total carbohydrate (including crude fibre and starch). There is variation in the nutritional composition of BGN which could explain the variation in starch content, reported in this work and that of Adebowale (2002).

4.4 Effect of BGNF and BGNS on Oil-Water Emulsion

4.4.1 Effect of oil and BGNF on the migration rate and droplet size of O/W emulsion

Table 4.1 provides the details of the effect of BGNF and oil concentration on migration rate (MR) and droplet diameter of BGNF stabilized emulsions on the first day. The prevalent destabilization phenomenon found in oil-in-water emulsions stabilized with BGNF was creaming as shown in the reference mode of turbiscan profiles in Figures 4.2 and 4.3. This was observed by the shift between 0-10 mm zone of the tube and therefore was used for the emulsion stability analysis. Similar trends in the BS% profiles have been used by Palazolo et al. (2004) to study the destabilization kinetics of the o/w emulsions. Destabilization phenomenon by creaming (encircled at the right hand side of the graph) manifested as an increase in the backscattering flux at the top of the sample. This is normally due to the migration of the oil particles from the bottom to the top of the turbiscan

Figure 4.1 Bambara groundnut flour (a) and Bambara groundnut starch (b)

Table 4.1 Migrate rate and droplet diameter of oil-water emulsion containing BGNF

Sample	Flour (% w/v)[1]	Oil (% w/v)[2]	Migration rate(MR) (mm/min)[3]	Droplet diameter(μm)[3]
1	2	5	0.2350 ± 0.0040	0.0300 ± 0.0020
2	2	30	0.0370 ± 0.0020	0.0720 ± 00003
3	2	55	0.0580 ± 0.0015	0.0720 ±0.0003
4	6	5	0.1400 ± 0.0300	0.0120 ± 0.0001
5	6	30	0.0490 ± 0.010	0.0008 ± 0.0004
6	6	55	0.0140 ± 0.0200	0.0007 ± 0.0004
7	10	5	0.0350 ± 0.0030	0.0004 ± 0.0002
8	10	30	0.0043 ± 0.0002	0.0004 ± 0.0004
9	10	55	0.0059 ± 0.0002	0.0006 ± 0.0002

[1] Values represent the amount of flour gelatinized with sufficient water to make 100 mL

[2] Values represent the amount of oil added to the 100 g gelatinized flour

[3] Values are mean ± standard deviation of duplicate.

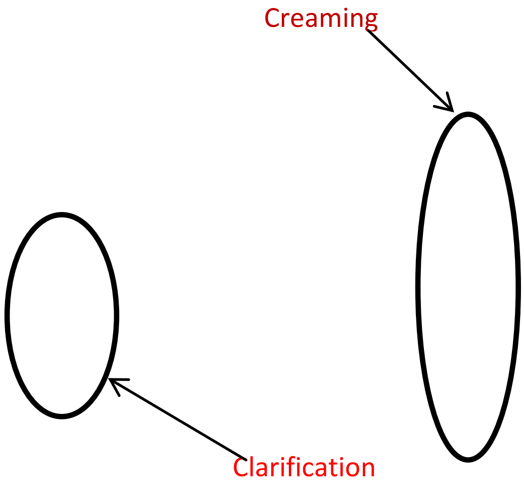


Figure 4.2 Turbiscan profile of emuslison containing 2% w/v BGNF and 55% w/v oil showing destabilization on day 1

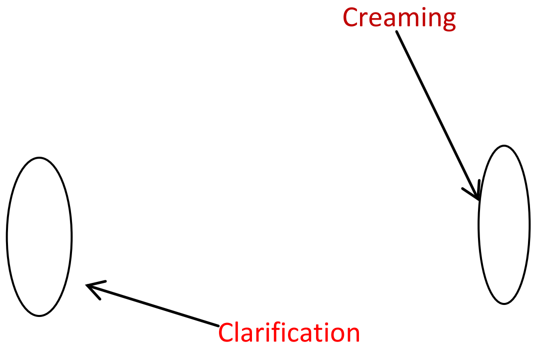


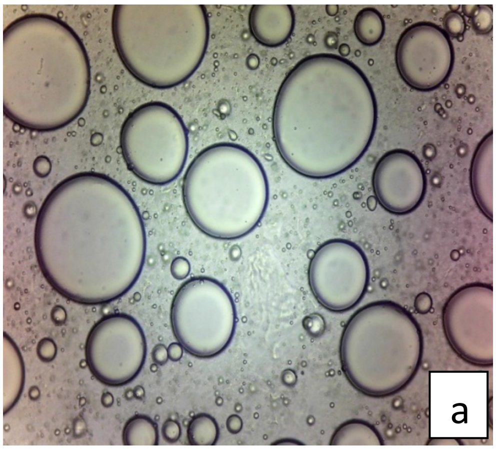
Figure 4.3. Profile for emulsion containing 10% w/v BGNF and 30% w/v oil showing relative instability

cell (Martini and Tippetts 2008). Other profiles of BGNF stabilized emulsions showing creaming destabilisation phenomenon are presented in Appendix 1.

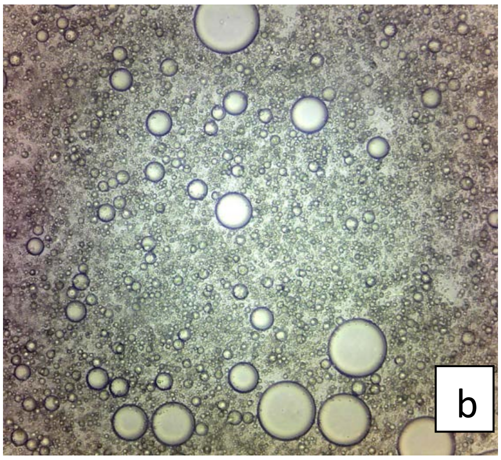
Emulsions with 2% w/v BGNF and varying amount of oil 5% w/v, 30% w/v and 55% w/v oil showed migration rates in increasing order: 0.2350 ± 0.0040 mm/min, 0.0300 ± 0.0002 mm/min, and 0.0580 ± 0.0015 mm/min respectively. This suggests that the higher the oil concentration, the higher the instability. It may also suggest that the concentration of the emulsifier was too low to emulsifying such oil:water proportion thereby encouraging creaming, which is the movement of the oil droplets from the bottom of the turbiscan tube to the top (Figure 4.2). Furthermore, the lowest values of MR (0.0043 ± 0.0002 mm/min, 0.0004±0.0004 mm/min and 0.0006 ± 0.0002 mm/min), were observed in the emulsion with highest BGNF (10% w/v). When compared within the group (10% w/v BGNF, and varying oil concentration – 5% w/v, 30% w/v and 55% w/v), the most stable emulsion was produced by the lowest oil composition. This could be due to the fact that the concentration of the BGNF was high enough to hold the water and oil together resulting in slow migration of the droplet particles (Figure 4.3). This was in agreement with the report of Mezdour et al (2008) that the presence of hydroxypropyl cellulose (HPC) was required for improved stabilization against creaming in emulsions stabilized with lecithin. The HPC chain acts strictly in other to improve the emulsion stability however both the lecithin and HPC must be in a right mixture because both compounds compete for the same interface.

In general, the emulsion MR ranges from 0.2350 ± 0.0040 mm/min for the lowest BGNF (2% w/v) to 0.0043 ± 0.0002 mm/min for the highest BGNF (10% w/v) concentration. This implies that the higher the BGNF concentration, the more stable the emulsion. Similar trend was observed with the droplet diameter. Emulsions with the lowest BGNF concentration (2% w/v) but varying amount of oil have the highest ranges of droplet diameters 0.0300 ± 0.0020 µm to 0.0720 ± 0.00030 µm. The lowest droplet diameter of 0.0004 ± 0.0004 µm was obtained from the emulsion with highest BGNF concentration (10% w/v) report of Achouri et al. (2012) surported the observation that increased oil content can lead to increased average oil droplet size. Also, Nor Hayati et al. (2009) showed that emulsion stability increased with the presence of some polysacchardes and most of the polysaccharides involved have some traces of suface activity which can hinder emulsion droplets from coalescing and thereby preventing creaming. Hence, BGNF can be seen as polysaccharide having surface activitity due to the presence of protein. The photomicrographs presented in Figure 4.4 and the graphical representation (Figure 4.5) show the decreasing droplet size with increasing concentration of BGNF.

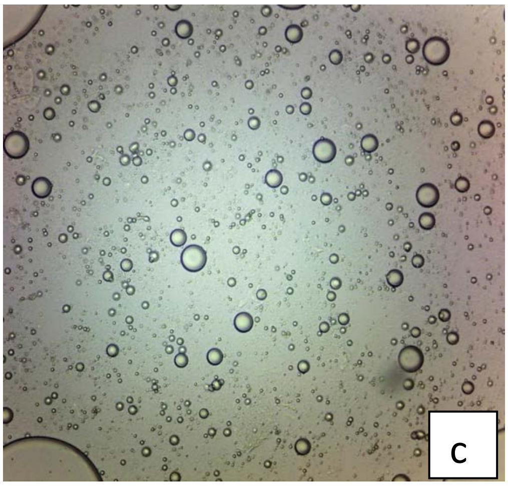
Similar observation was reported by Zinoviadou et al. (2012) in their work to determine the properties of emulsions stabilized by sodium caseinate-chitosan complex. They reported that emulsions stabilized by these complexes with increased levels of chitosan (>0.2% w/w) had a smaller average droplet size and exhibited greater stability during storage.



2% w/v BGNF

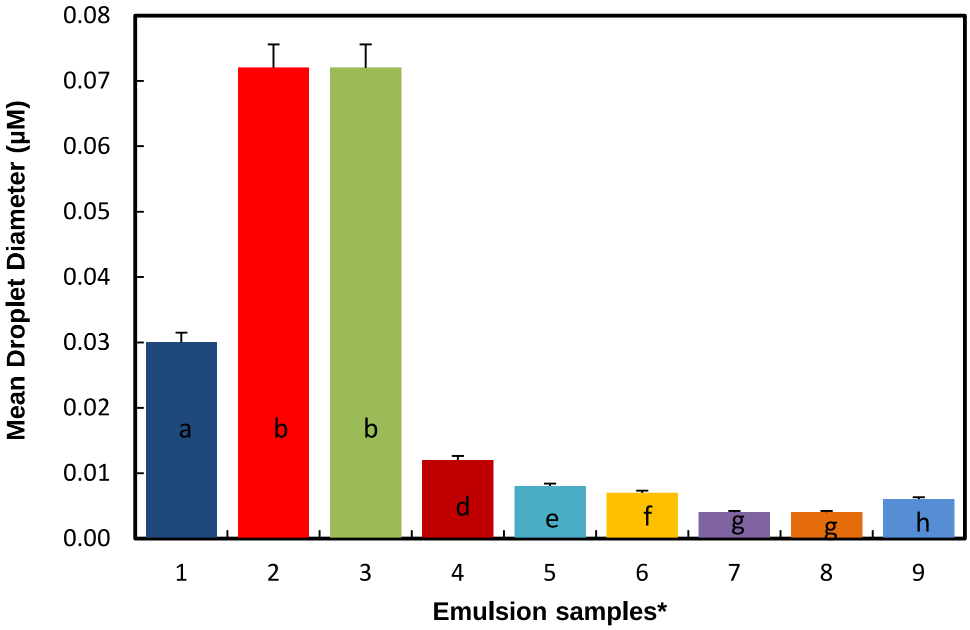


6% w/v BGNF



10% w/v BGNF

Figure 4.4 The day 1 photomicrograph of BGNF-stabilized emulsions containing similar oil concentration but with increasing BGNF concentration of (a) 2% w/v; (b) 6% w/v; and (c) 10% w/v



**Bars with different letters are significantly different (p < 0.05)*

Figure 4.5 The absolute mean droplet sizes of the BGNF-stabilized emulsions as observed on Day 1.
1 = 2% w/v + 5% w/v oil; 2 = 2% w/v BGNF + 30% w/v oil; 3 = 2% w/v BGNF 55% w/v oil; 4 = 6% w/v BGNF + 5% w/v oil; 5 = 6%; BGNF + 30% w/v oil; 6 = 6% w/v BGNF + 55% w/v oil; 7 = 10% w/v BGNF + 5% w/v oil; 8 = 10% w/v BGNF + 30% w/v oil; 9 = 10% w/v BGNF + 55% w/v oil.

Response surface model for BGNF emulsion

The analysis of variance (ANOVA) for quadratic polynomial model for emulsion migration rate (Table 4.1) is given in Table 4.2. The lack of fit was not significant ($p > 0.05$); high R^2 value of 0.996 and high adjusted R^2 value of 0.990 indicate that the quadratic polynomial model adequately explained the variation in the MR (Karazhiyan et al., 2011). Hence, the model was adequate to explore the design space. The main effect of flour and oil was significant ($p < 0.05$) on the migration rate; the quadratic effect of oil was significant ($p < 0.05$) on migration rate. However, the quadratic effect of flour was not significant ($p > 0.05$). There was significant ($p < 0.05$) interaction between the flour and oil on migration rate.

The effects of flour and oil were negative on the migration rate. This means that increasing oil or flour decreased migration rate. In other words, they cannot stabilise emulsion singly, but the interaction of both flour and oil including their quadratic effect have positive effect on the migration rate. They both encouraged emulsion stability by lowering the migration rate and this can be seen from Figure 4.6 where the increase in flour and oil led to decrease in the migration rate. The relationship between the BGNF and oil with respect to migration rate is given by equation 4.1

$$MR = 0.3315 - 0.0332\,\mathrm{X}_1 - 7.5222 \text{ x } 10^{-3}\mathrm{X}_2 + 2.6571 \text{ x } 10^{-4} X_1X_2 + 1.0111 \text{ x}10^{-3} X_1^2 + 5.7371 \text{ x } 10^{-5} X_2^2 \tag{4.1}$$

where MR = migration rate (mm/min), X_1 = BGNF (% w/v), X_2 = Oil (% w/v), X_1X_2 = interactive effects of BGNF and oil, X_1^2 = quadratic effect of BGNF, X_2^2 = quadratic effect of oil.

Optimum BGNF for a stable emulsion

Numerical optimisation was used to estimate the BGNF and oil concentration that will produce minimum migration rate (Design Expert 8). The emulsion with 9% w/v BGNF and 39% w/v oil with desirability produced the optimal emulsion. This result was verified by producing the emulsion with 9% w/v BGNF and 39% w/v oil. The migration rate of the optimal emulsion was very low and physically-observed stable.

Table 4.2 Analysis of variance (ANOVA) for the effect of BGNF and oil on the emulsion migration rate

Source	Sum of squares	df	Mean square	F-value	p-value
Model	0.071	5	0.014	186.92	< 0.0001
Flour	7.520E-003	1	7.520E-003	99.00	0.0006
Oil	0.012	1	0.012	164.24	0.0002
Flour * Oil	1.561E-003	1	1.561E-003	20.55	0.0106
$Flour^2$	5.084E-004	1	5.084E-004	6.69	0.0609
Oil^2	1.588E-003	1	1.588E-003	20.91	0.0102
Residual	3.039E-004	4	7.596E-005		
Lack of fit	3.857E-006	1	3.857E-006	0.039	0.8569
Pure error	3.000E-004	3	1.000E-004		
Cor Total	0.071	9			
R^2	0.9957				
Adj R^2	0.9904				
C.V%	10.45				

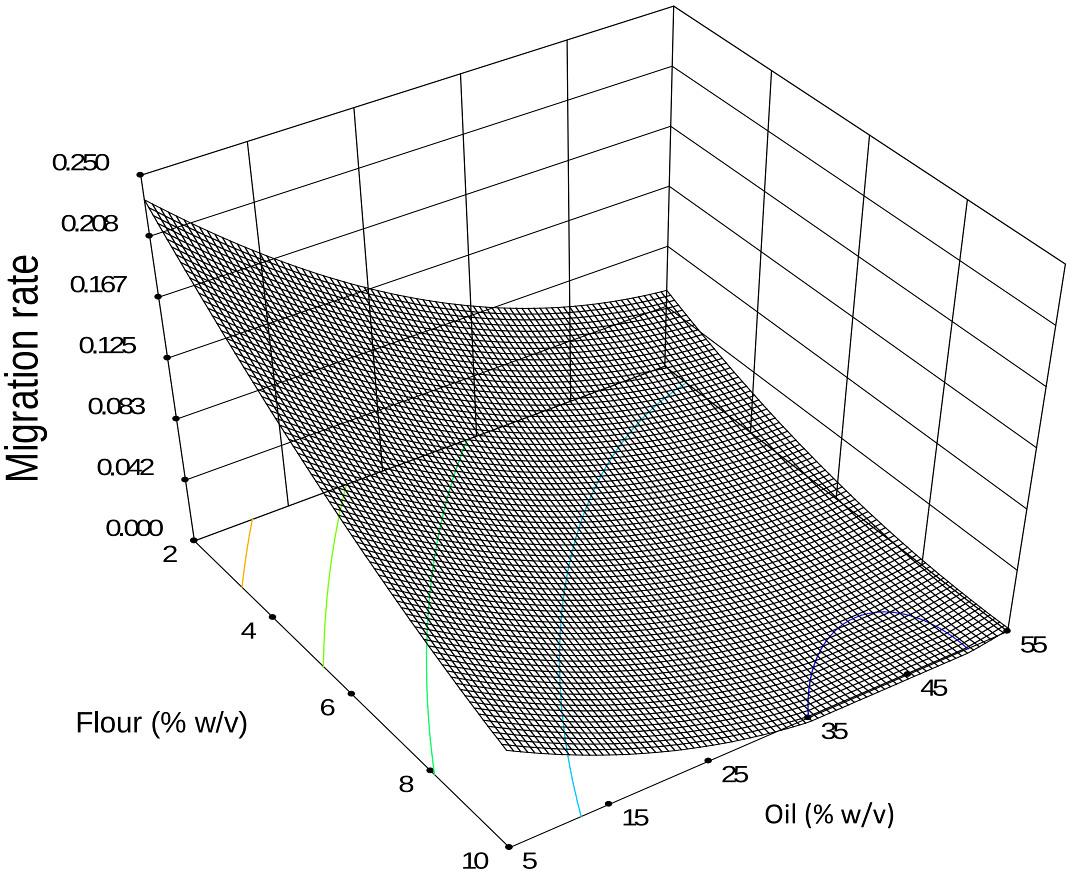


Figure 4.6 The effect of BGNF and oil concentration on the droplet migration rate of the BGNF-stabilized emulsions

4.4.2 Effect of oil and BGNS on the migration rate (MR) and droplet size of BGNS emulsion

The effect of BGNS on the MR and droplet size is shown in Table 4.3. The MR ranges from the highest value of 0.4400 ± 0.0150 mm/min for the emulsion with the lowest amount of BGNS (1% w/v) and highest oil (55% w/v) to the lowest MR value of 0.0021 ± 0.0003 mm/min for the emulsion containing the highest amount of BGNS (5% w/v) and higher amount of oil (30% w/v). The representative Turbiscan® profiles of oil-in-water emulsions stabilized by BGNS are as presented in Figures 4.7 and 4.8. It was observed that O/W emulsions stabilized with BGNS showed multiple destabilization phenomena. The shift between 0-10 mm was an indicative of destabilization through creaming phenomenon. Creaming is the movement of oil-droplets from the bottom to the top of the tube leaving a clarified zone between 0-10 mm. This phenomenon can be identified as depletion (clarification) at the bottom (encircled left hand side of the graph) of the tube and an overshoot in the backscattering flux at the top (encircled right hand side of the graph) of the tube (Martini and Tippetts 2008).

Sedimentation phenomenon of the oil particles showed as an increase in the backscattering flux at the bottom on the left hand side of the graph (Chauvierre et al., 2004).

The third zone, which is the centre of the Turbiscan® graph, is attributed to destabilization through aggregation of the oil droplets (flocculation/ coalescence). This is showed by the decrease in the backscattering flux on the whole height of the sample (horizontal axis of the Turbiscan® graph) (Chauvierre et al., 2004).

However, the creaming kinetics was used for the analysis of emulsion stability because of the consistency and it was the most observed destabilization phenomenon relative to others.

The highest droplet size of 0.0700 ± 0.0003 µm was observed in the emulsion containing the lowest amount of BGNS (1% w/v) and the highest amount of oil. In the same way, the lowest droplet size of 0.0100 ± 0.0002 µm was found in the emulsion with the highest BGNS concentration (5% w/v) and the higher amount of oil (30% w/v). Therefore, instability of the emulsions increased with decreasing BGNS concentration, and increasing amount of oil. Figure 4.9 shows the effects of increasing BGNF concentration on droplet size With droplets size ranges from 0.0240 ± 0.0002 (µm) to 0.0700 ± 0.0003 (µm) for emulsions containing the same amount of BGNS (1% w/v) with different amount of oil (5%, 30%,55% w/v) respectively and this trend was the same for all the emulsions prepared with BGNS. Figure 4.10 provides graphical profiles of the absolute mean droplet sizes with time for BGNS-stabilized emulsions.

Table 4.3 Migration rate and droplet diameter of oil-water emulsion containing BGNS

Sample	Starch(% w/v)[1]	Oil (% w/v)[2]	Migration rate (MR) (mm/min)[3]	Droplet diameter (µm)[3]
1	1	5	0.1250 ± 0.0015	0.0240 ± 0.0002
2	1	30	0.1180 ± 0.0020	0.0254 ± 0.0003
3	1	55	0.4400 ± 0.0150	0.0700 ± 0.0003
4	3	5	0.0180 ± 0.0020	0.0130 ± 0.0004
5	3	30	0.0605 ± 0.0005	0.0380 ± 0.0004
6	3	55	0.1595 ± 0.0030	0.0450 ± 0.0004
7	5	5	0.0066 ± 0.0003	0.0110 ± 0.0001
8	5	30	0.0021 ± 0.0003	0.0100 ± 0.0002
9	5	55	0.0088 ± 0.0004	0.0120 ± 0.0003

[1] Values represent the amount of Starch gelatinized with sufficient water to make 100 mL

[2] Values represent the amount of oil added to the 100 mL gelatinized flour

[3] Values are mean ± standard deviation of duplicate

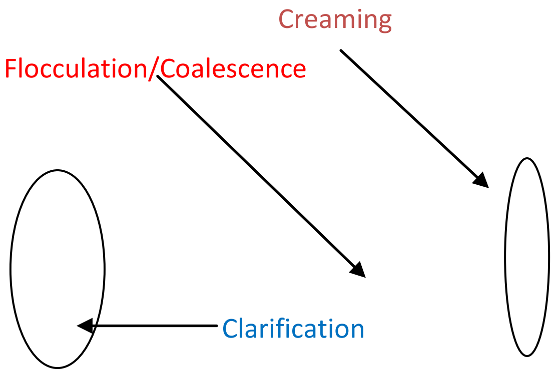


Figure 4.7 Turbiscan profile showing destabilization kinetics in 1% w/v BGNS and 5% w/v oil

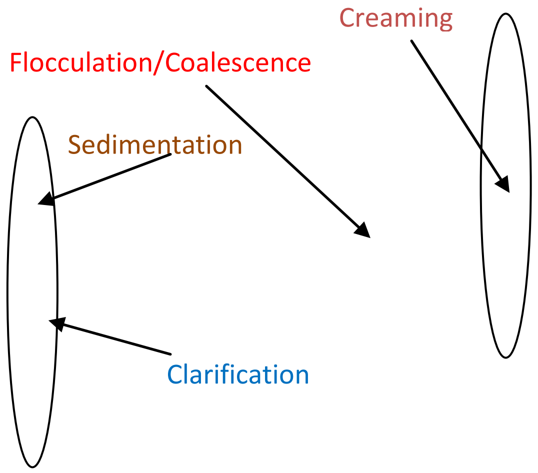


Figure 4.8 Turbiscan profile showing destabilization kinetics in 5% w/v BGNS and 55% w/v oil

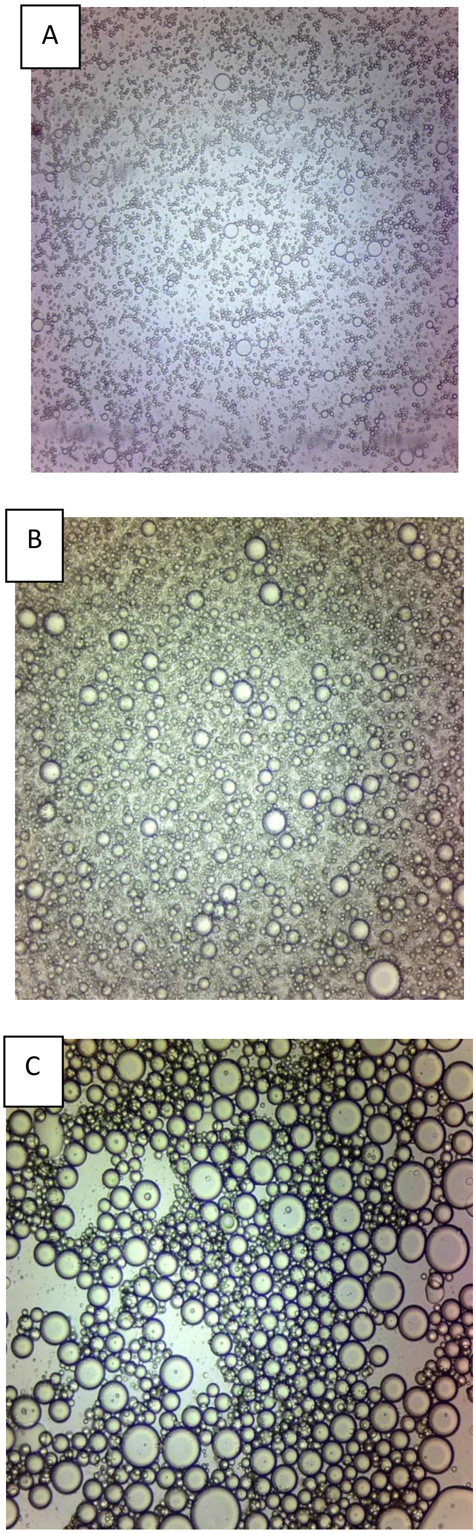
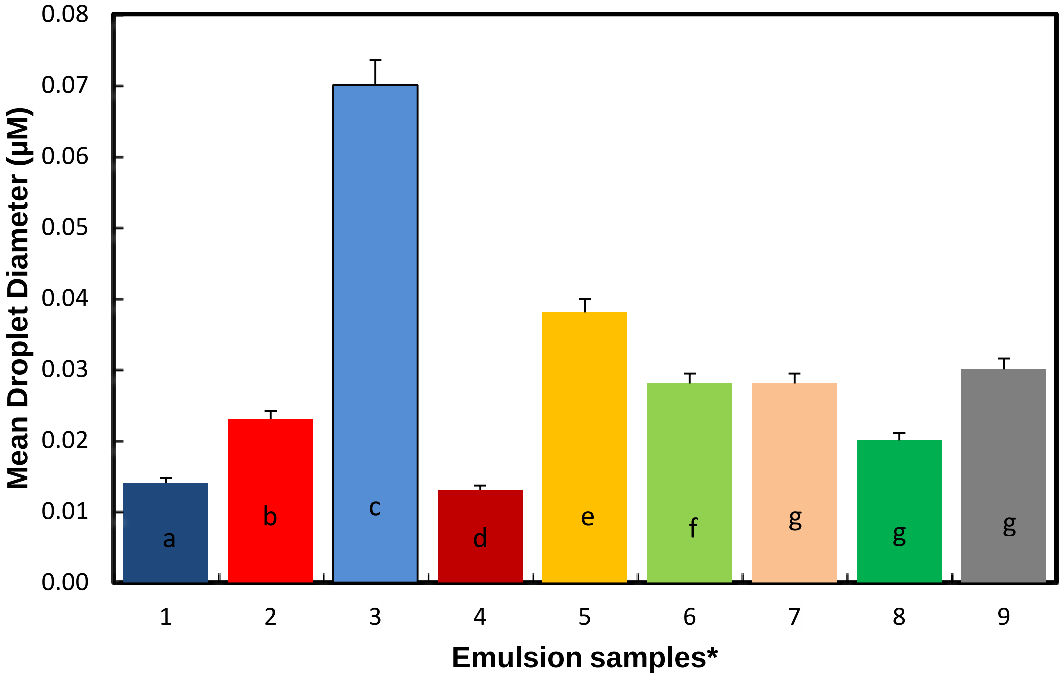


Figure 4.9 Photomicrographs of BGNS-stabilized emulsions with (a) concentrated o/w emulsions showing flocculation; (b) growth in droplet diameter from (a); and (c) visible signs of phase separation



Bars with different letters are significantly different ($p < 0.05$)

Figure 4.10 The absolute mean droplet sizes of the BGNS-stabilized emulsions as observed on Day 1.

1 = 1% w/v BGNS +5% w/v oil; 2 = 1% w/v BGNS + 30% w/v oil; 3 = 1% w/v BGNS + 55%% w/v; 4 = 3% w/v BGNS + 5% w/v oil; 5 = 3% w/v BGNS + 30% w/v oil; 6 = 3% w/v BGNS + 55% w/v oil; 7 = 5% w/v BGNS + 5% w/v oil; 8 = 5% w/v BGNS + 30% w/v oil; 9 = 5% w/v BGNS + 55% w/v oil.

Response Surface Model for BGNS emulsion

Table 4.3 provided the detail of BGNS-stabilized oil-in-water emulsion composition of the emulsions. The analysis of variance (ANOVA) for quadratic polynomial models for emulsion migration rate is given in Table 4.4. The insignificant ($p > 0.05$) lack of fit in addition to the high R^2 value of 0.996 and high adjusted R^2 value of 0.990 indicate that the quadratic polynomial model adequately explained the variation in the MR. Hence, the model was adequate to explore the design space. The main effect of starch and oil was significant ($p < 0.05$) on the migration rate, the quadratic effect of oil as well as starch were also significant ($p < 0.05$) on migration rate. There was significant ($p < 0.05$) interaction effect of starch and oil on migration rate.

The relationship between the BGNS and oil with respect to migration rate is given by equation 4.2.

$$MR = 0.13358 - 0.068905\,\mathrm{X}_1 + 4.97721 \times 10^{-3}\mathrm{X}_2 - 1.34625 \times 10^{-3} X_1X_2 + 9.17156 \times 10^{-3}X_1^2 + 3.04640 \times 10^{-5} X_2^2 \qquad (4.2)$$

where MR = migration rate (mm/min), X_1 = BGNS (% w/v), X_2 = oil (% w/v), X_1X_2 = interactive effects of BGNS and oil, X_1^2 = quadratic effect of BGNS, X_2^2 = quadratic effect of oil. The equation (4.2) gives the details of the impact of each emulsion variables. Increasing BGNS decreased migration rate whereas increase in oil increases migration rate significantly ($p < 0.05$). Their interactive effect was negative which means a little increase in either of the two will have greater negative effects on the migration rate. Hence, the quadratic effect is positive on the migration rate. Both encourage emulsion stability by lowering the migration rate and this can be seen from Figure 4.11.

Table 4.4 Analysis of variance (ANOVA) for the effect of Bambara groundnut starch and oil on the emulsion migration rate

Source	Sum of squares	DF	Mean square	F-value	p-Value prob F
Model	0.25	5	0.050	604.41	0.0001
Starch	0.079	1	0.079	959.70	0.0001
Oil	0.033	1	0.033	400.76	0.0001
Starch * oil	0.032	1	0.032	391.52	0.0001
Starch^2	4.554E-003	1	4.554E-003	55.24	0.0007
Oil^2	6.042E-004	1	6.042E-004	7.334	0. 0424
Residual	4.123E-004	5	8.245E-005		
Lack of Fit	2.058E-004	2	1.029E-004	1.49	0.3545
Pure Error	2.065E-004	3	6.883E-005		
Cor Total	0.25	10			
R^2	0.9983				
Adj R^2	0.9967				
C.V	7.02				

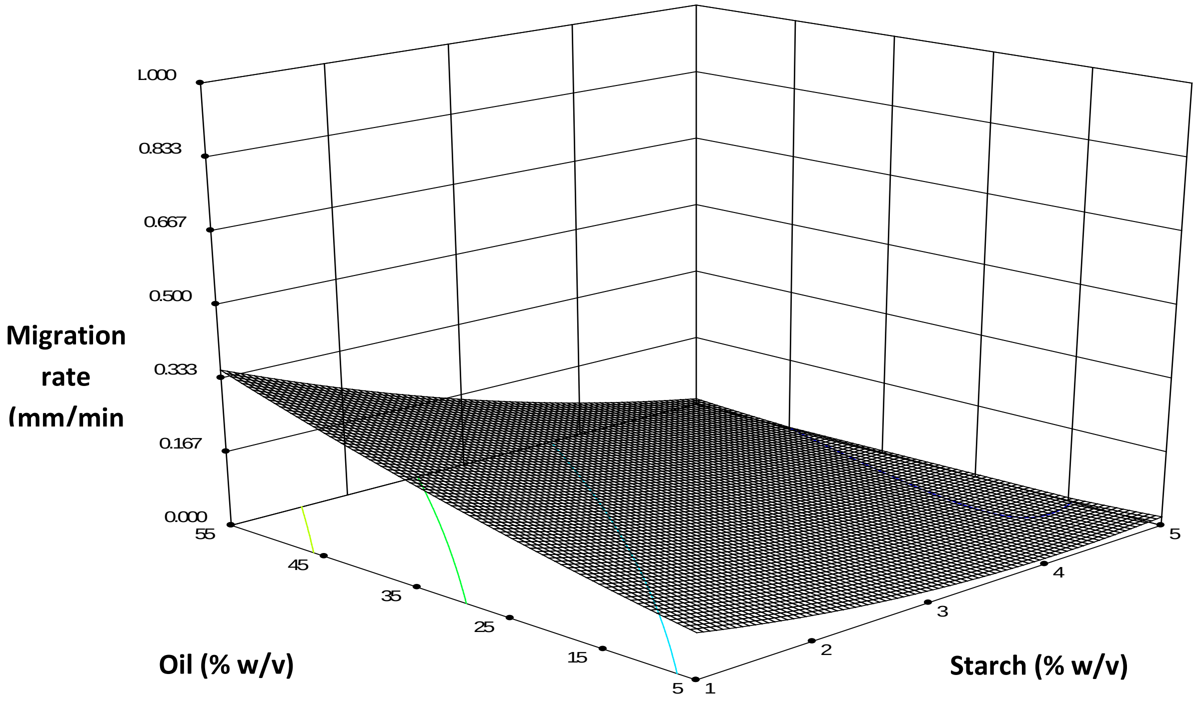


Figure 4.11 The effects of BGNS and oil concentration on the droplet migration rate of the BGNS-stabilized emulsion.

Optimum BGNS for a stable emulsion

The numerical optimisation with objectives to minimise migration rate in the range of starch and oil concentration used, was carried out. The emulsion with 5% w/v BGNS and 30% w/v oil having demonstrated the desirable properties was selected as the optimal.

In general, the MR obtained for the BGNS stabilized emulsion (0.0021 ± 0 .0003 mm/min to 0.4400 ± 0.0150 mm/min) was higher than the corresponding BGNF emulsion also, the mean droplet sizes of the BGNS-stabilized emulsions (0.100 ± 0.0002 µm to 0.0700 ± 0.0003 µm) are much higher than the corresponding BGNF-stabilized emulsions. The MR and mean droplet size values were significantly different ($p < 0.05$) in both cases. BGNF thus, appears to be a better emulsifier/stabilizer. This may be related to the additional protein and ash composition of BGNF, which are absent in the BGNS. Much of the reported works on emulsifying capacity of polysaccharides can be explained in terms of complexation or contamination with a small fraction of surface active protein. In mixed protein and polysaccharide systems, associative electrostatic interactions can lead to coacervation or soluble complex formation depending on the nature of the biopolymers and the solution conditions (Dickinson, 2003). McClements (2006) also reported that either attractive or repulsive interactions between proteins and polysaccharides can also help with emulsion stability.

4.5 Effects of Storage Time on the Optimized BGNF and BGNS-Stabilised Emulsions

4.5.1 Effects of storage time on the MR and droplet size of the BGNF-stabilized emulsion

Storage time had no significant ($p > 0.05$) effect on the MR of BGNF-stabilised emulsions but the droplet size was significantly ($p < 0.05$) different (Table 4.5). The storage time had significant ($p < 0.05$) increase on the droplet size but not on the MR (Tables 4.5 and 4.6).

The mean migration rate (MR) of the emulsions stabilised with BGNF was observed to be 0.0134 ± 0.0039 mm/min on day 1 with the least MR, while the highest MR value was observed on day 5 with the mean MR value of 0.0283 ± 0.0042 mm/min. The effect of storage on the MR on day 2 with mean MR value, 0.0188 ± 0.0389 mm/min was not significantly ($p > 0.05$) different from that observed on day 1. The MR of the emulsion increased steadily to a threshold of 0.0349 ± 0.0389 mm/min on day 4, after which the MR decreased by 19% to 0.0283 ± 0.0424 mm/min. Thus, the MR response to emulsion properties between day 1 and day 3 appeared consistent. However, the change in MR observed on day 4 may be as a result of intermolecular/intermolecular changes in emulsion composition with storage duration (Figure 4.12, 4.13, 4.14, 4.15 and 4.16).

Table 4.5 Tests of Between-Subjects Effects for migration rate of BGNF emulsions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.001[b]	4	0.000	3.752	0.090
Intercept	0.006	1	0.006	156.596	0.000
day	0.001	4	0.000	3.752	0.090
concentration	0.000	0	.		

[a] R Squared = 0.750 (Adjusted R Squared = 0.550)

Table 4.6 Tests of Between-Subjects Effects for droplet sizes of BGNF emulsions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.581E-005[b]	4	3.952E-006	11.831	0.009
Intercept	.000	1	0.000	1049.293	0.000
day	1.581E-005	4	3.952E-006	11.831	0.009
concentration	.000	0	.	.	.

[a] R Squared = 0.904 (Adjusted R Squared = 0.828)

Figure 4.12 Samples of the BGNF-stabilized emulsions, physically stable on day 1

Figure 4.13 Samples of the BGNF-stabilized emulsions, physically stable on day 5

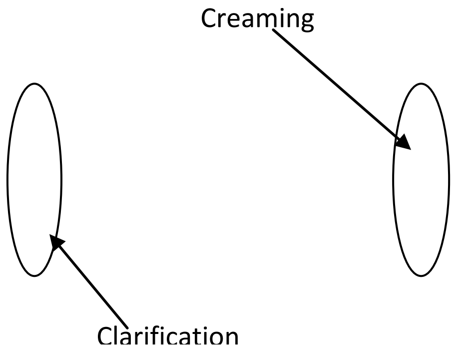


Figure 4.14 Tubiscan® destabilisation profile for BGNF optimal emulsion on day 1

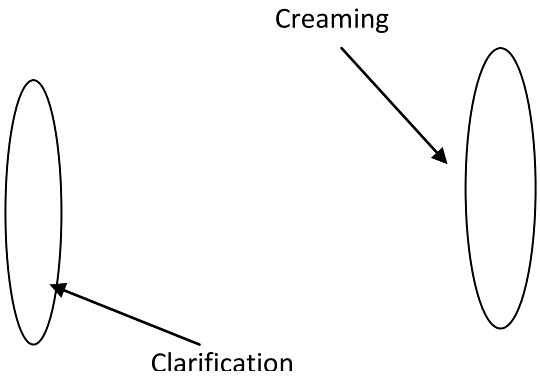


Figure 4.15 Tubiscan® destabilisation profile for BGNF optimal emulsion on day 5

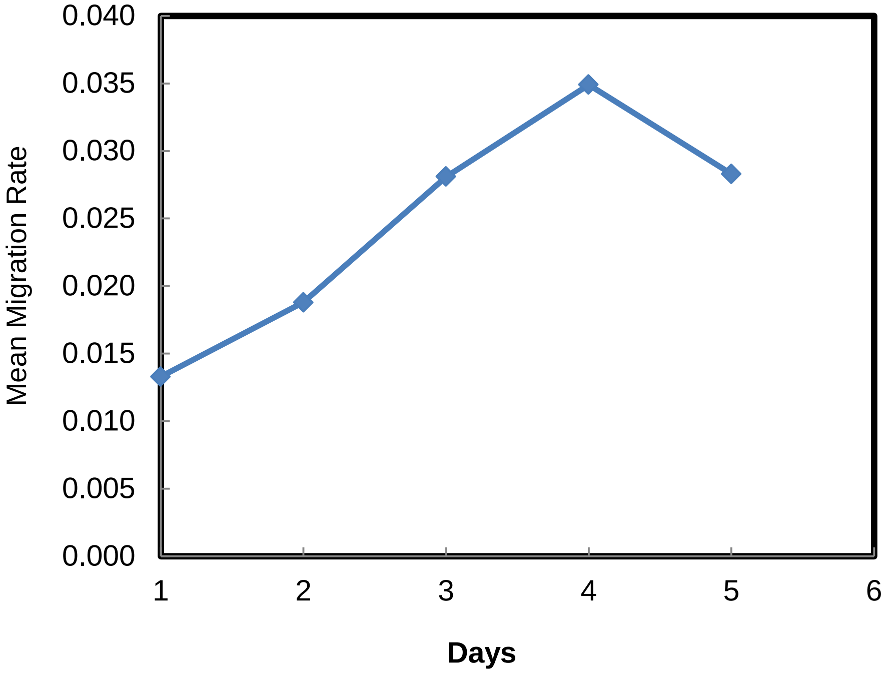


Figure 4.16 The mean migration rate of the emulsion containing 9% w/v BGNF and 39% w/v oil as affected by storage time

Figures 4.17 and 4.18 show the growth of the droplet size of the optimal emulsion containing 9% w/v BGNF and 39% w/v oil. The particle size growth over the days was minimal and the combination provided an optimal oil and water stabilized by BGNF.

As shown in Figure 4.18, the droplet size was least on day 1 (0.0385±0.0021 µm) and highest on day 5 with droplet size 0.0075 ± 0.00070µm, while the overall mean droplet size over the storage duration period was 0.0592 ± 0.0139 µm. There appeared to be a linear response to the increase in the droplet size of the BGNF-stabilized emulsion with increased storage duration. This explains the high R^2 of 0.904 obtained for the correlation. The value observed on day 1 storage was not significantly different ($p > 0.05$) from that measured on day 2. Also the droplet size obtained on day 2, day 3 and day 4 were also not significantly different ($p > 0.05$). The droplet size noticed on day 3, day 4 and day 5 were also not significantly different ($p > 0.05$). However, droplet size on day 1 increased significantly ($p < 0.05$) to day 5.

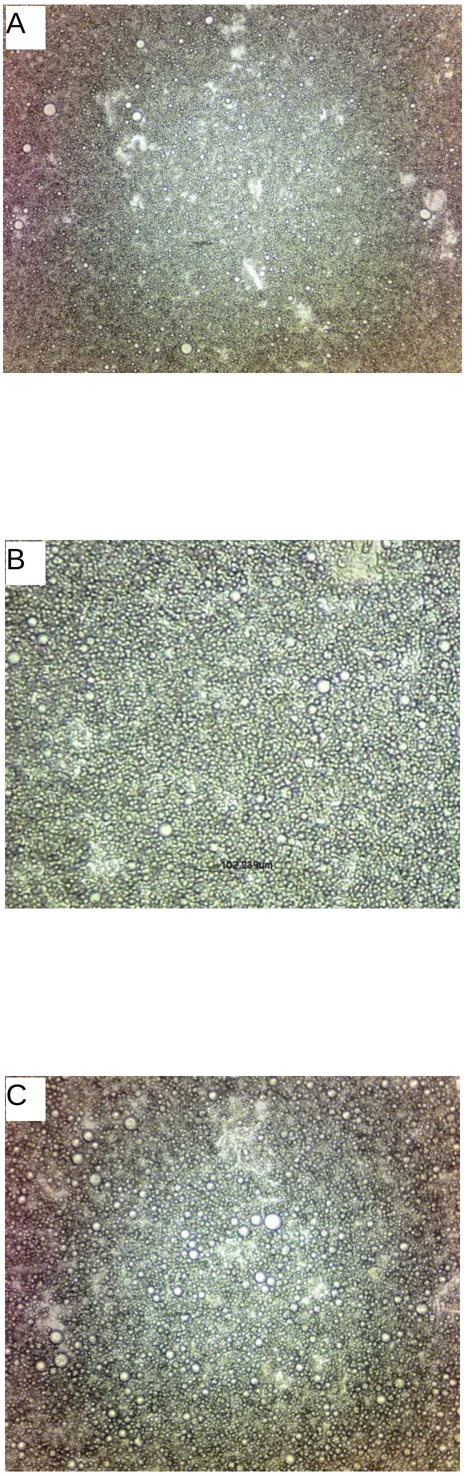
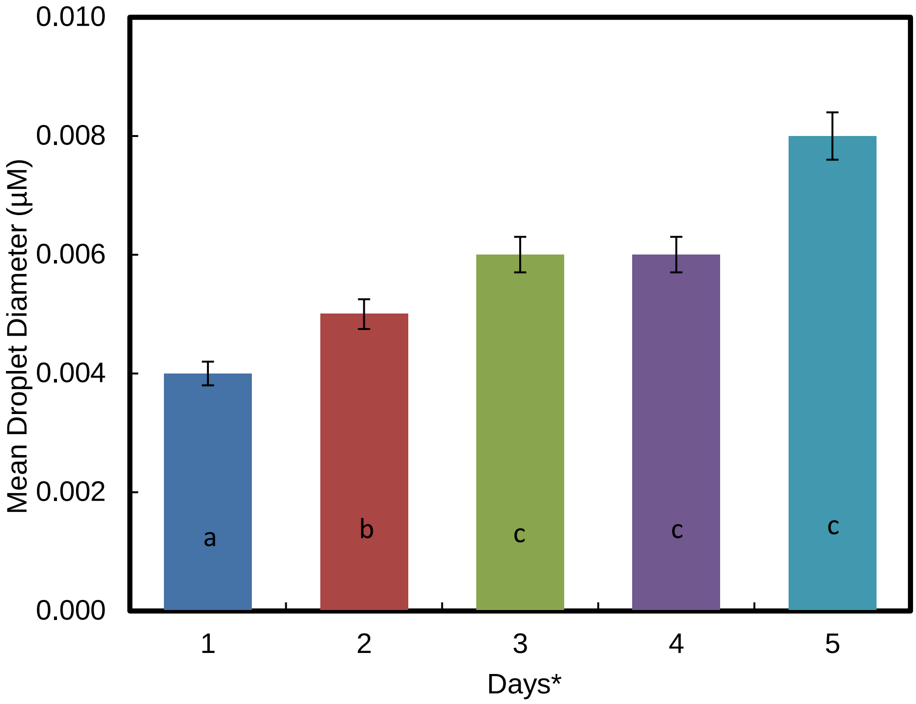


Figure 4.17 Increase in oil droplet size of the optimal BGNF-stabilized emulsion for a) day 1, b) day 3 and c) day 5



**Bars* with the same letters are not significantly different (p > 0.05).

Figure 4.18 The absolute mean droplet sizes of the emulsion containing 9% w/v BGNF and 39% w/v oil as affected by storage time

The increase in the MR (0.0134 ± 0.0039 mm/min) and the mean droplet size 0.0385 ± 0.0021 µm on day 1 to the MR value (0.0283 ± 0.0042) and mean droplet size (0.05920 ± 0.00139 µm) on day 5 were not significantly ($p > 0.05$) different with storage time. This fits into the hypothesis that the BGNF stabilized emulsions are stable with storage time and this is in agreement with the result reported by Hayati et al. (2007) that soya bean oil/water emulsion stabilised with egg yolk showed increased stability with storage. Most experimental emulsions destabilize in hours but BGNF-stabilized emulsions were stable for 5 days when they were discarded despite the absence of any co-stabilizer. The trend of destabilization suggested that the emulsions will survive for longer days. Future work should be aimed at incorporating a preservative into the emulsion to study the stability beyond the duration used in this work.

4.5.2 Effects of storage time on the MR and droplet size of the BGNS-stabilized emulsion

On physical examinations, the emulsions appeared generally stable up to 5 days (Figure 4.17). On analysis, storage time had significant ($p < 0.05$) effect on the MR and droplet size of BGNS-stabilised emulsions (Tables 4.7 and 4.8). The storage time showed significant ($p < 0.05$) increase in the MR and droplet size. This indicates that the influence of storage time differs. Traynor et al. (2013) also reported a significant effect of xanthan gum and lecithin on emulsion stability and mean droplet diameter over a storage period of 14 days.

The MR of this BGNS-stabilized emulsion observed on day 1 was significantly different ($p < 0.05$) from the MR value of the emulsion after day 2. The MR for storage duration between day 2 to day 5 were however not significantly different ($p > 0.05$) from one another (Figure 4.19 and 4.20). Thus, the storage time has no significant ($p > 0.05$) effects on the BGNS stabilized emulsion from day 2 to day 5 (Figure 4.21).

The particle sizes were generally higher than those observed in BGNF-stabilized emulsions. The mean droplet size of BGNS-stabilized emulsion increased over the storage duration from 0.0195 ± 0.0070 µm to 0.0340 ± 0.0028 µm with an overall mean of 0.027 ± 0.0055 µm over the storage duration (Figure 4.22 and 4.23). There was cluster homogeneity in droplet size within inter-day storage period. For instance, there was no significant ($p > 0.05$) difference between the droplet size obtained for starch droplets after day 1 and day 2 storage; day 2 and day 3 storage; day 3 and day 4 storage; and day 4 and day 5 storage (Figure 4.23). However, the droplet size were significantly ($p < 0.05$) different over an extended period of 48 hr i.e. between day 1 and day 3; day 2 and day 4; and day 3 and day 5. The rate of instability on the subsequence storage days was higher than those observed in BGNF-stabilized emulsions.

Table 4.7 Tests of Between-Subjects effects for droplet sizes of BGNS emulsions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.002[b]	4	.000	6.601	.031
Intercept	.014	1	.014	222.092	.000
day	.002	4	.000	6.601	.031
Starch	.000	0	.	.	.
Error	.000	5	6.174E-005		
Total	.016	10			
Corrected Total	.002	9			

[a] R Squared = .841 (Adjusted R Squared = .713)

Table 4.8 Tests of Between-Subjects effects for droplet sizes of BGNS emulsions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.000[b]	4	6.375E-005	13.859	.006
Intercept	.007	1	.007	1584.783	.000
day	.000	4	6.375E-005	13.859	.006
Starch	.000	0	.	.	.
Error	2.300E-005	5	4.600E-006		
Total	.008	10			
Corrected Total	.000	9			

[a] R Squared = .917 (Adjusted R Squared = .851)

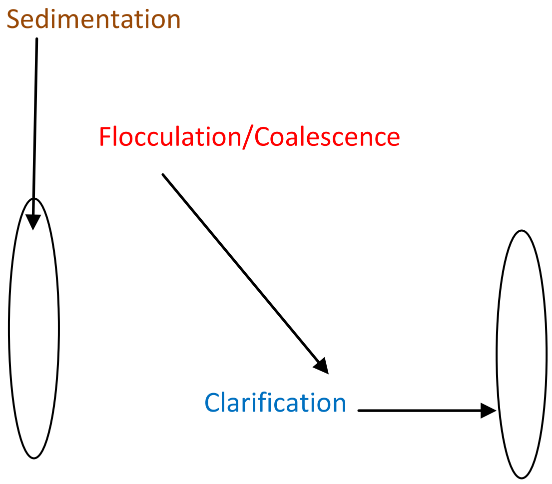


Figure 4.19 Turbiscan profile shown destabilization kinetics of the optimal BGNS on day 1

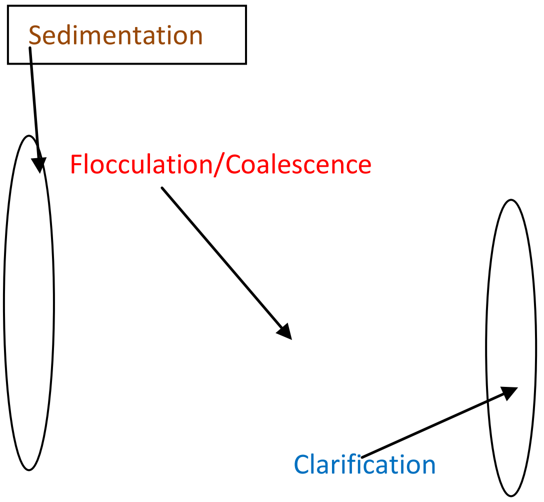


Figure 4.20 Turbiscan profile showing destabilization kinetics of the optimal BGNS-stabilized emulsion on day 5

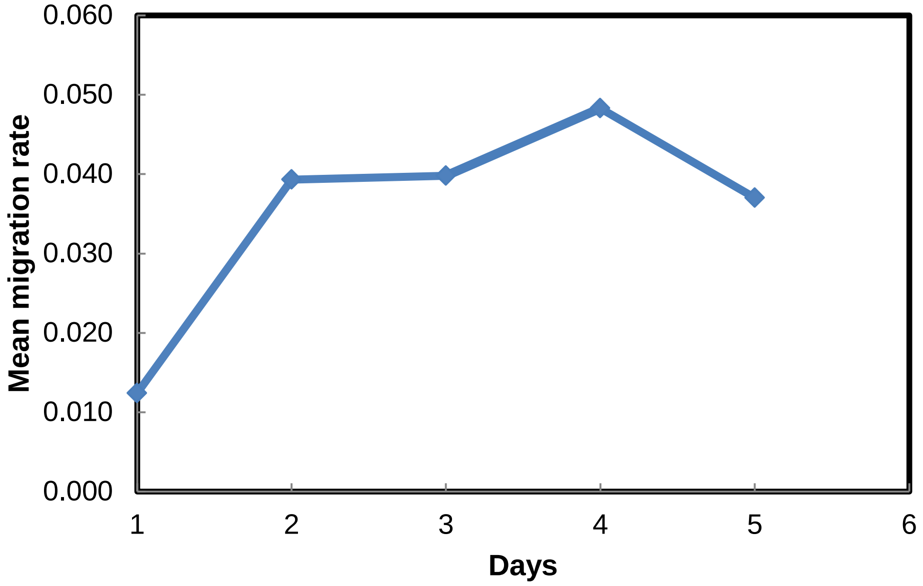


Figure 4.21 The mean migration rate of the emulsion containing 5% w/v BGNS and 30% w/v oil as affected by storage time.

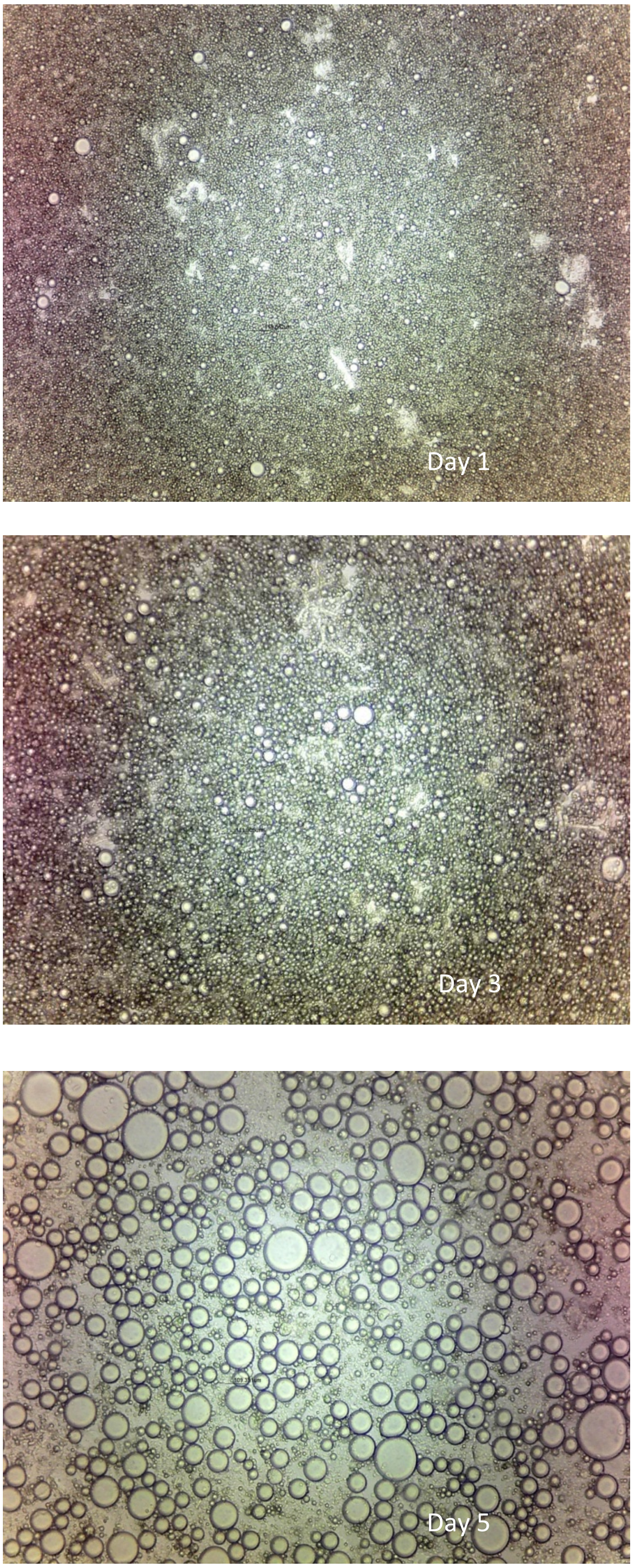
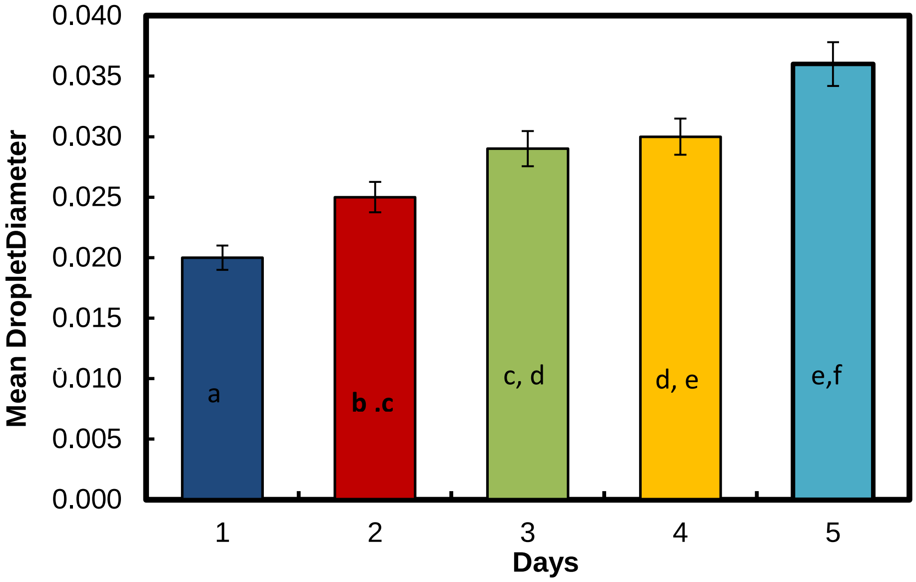


Figure 4.22 Increase in oil droplet size of BGNS-stabilized emulsion observed from day 1, through day 3 to day 5



*Bars with the same letters are not significantly different ($p < 0.05$)

Figure 4.23 The absolute mean droplet sizes of the emulsion containing 5% w/v BGNS and 30% w/v oil as affected by storage time

The current findings reveal the ability of BGN to stabilize emulsions generally agree with earlier literature reported Eltayeb (2011) reported that BGNF demonstrated water-absorption capacity of 281.35% while its protein isolate has 221.83% capacity; the protein isolate demonstrated 210% foaming capacity at pH 9.0, and emulsion stability of 70% after 48 h. Lawal et al. (2007) investigated the functional properties of native and chemically modified protein isolates of BGN. The results indicate an initial increase in emulsifying activity with increase in protein concentration. Both acetylation and succinylation improved the emulsifying stability of the native protein.

Although this emulsifying property was found with the protein isolate, the result of the current study suggests that the whole flour may be a better emulsifier than the isolated protein. Whole legume flours have demonstrated functional properties including emulsifying activity attributed to their dynamic protein and carbohydrate blend (Meng and Ma, 2002). Native starch has limited use in food products depending on the properties exhibited. Starches derivatives, also called modified starch are thus made through physical, enzymatic or chemical treatment of native starch in order to enhance their functional properties including improvement in texture, stability to temperature and pH. Such modification makes modified starches popular as thickening agents (viscosity modifiers), emulsifiers, tablet disintegrant and binder in paper industries. Thus, modified starch is used for example as toppings for pizza where it thickens upon heating and less viscous when cooled. Modified starch is also used as emulsifier in French dressing.

While native starch is seldom used as emulsifiers, a number of patented works have shown the good emulsifying properties of modified starch (Chiu, 1990; 1993). In the current study, the BGNF-stabilized emulsions generally demonstrated double the stability of the BGNS-stabilized ones. There are good reasons to suggest that the Protein component of the BGNF contributes significantly to the emulsifying superiority of BGNF. This is because, isolated BGN protein showed 70% emulsifying capacity in 48 hours in earlier study (Eltayeb 2011). BGNF however, has demonstrated higher emulsifying property, having stabilized emulsion for long (up to Day 5).

In summary, both BGNF and BGNS have demonstrated to varying degrees, the potential to emulsify emulsions with prospects for use in commercial food and pharmaceutical products.

4.6 Influence of Some Physico-Mechanical and Environmental Factors on the Optimal Emulsions

Similar multiple destabilization phenomena were observed for all physico-mechanical and environmental effects on the stability of the optimum emulsions (BGNF and BGNS stabilized). The following are the various destabilization mechanisms observed;

1 Creaming phenomenon which is the peak between 0 -10 mm. Creaming is the movement of oil-droplets from the bottom to the top of the tube leaving a clarified zone between 0-10 mm. This phenomenon can be identified as depletion (clarification) at the bottom (encircled left hand side of the graph) of the tube and an overshoot in the backscattering flux at the top (encircled right hand side of the graph) of the tube (Chauvierre et al., 2004).

2 Sedimentation phenomenon of the oil particles which showed as an increase in the backscattering flux at the bottom and decrease at the top (Chauvierre et al., 2004).

3 The third zone, which is the centre of the Turbiscan® graph, is attributed to destabilization through aggregation of the oil droplets (flocculation/ coalescence). This is showed by the decrease in the backscattering flux on the whole height of the sample (horizontal axis of the Turbiscan® graph) (Chauvierre et al., 2004).

However the creaming kinetics was used for the analysis of emulsion stability because of the consistency and it was the most observed destabilization phenomenon relative to others.

4.6.1 Stirring effects on the emulsions

Table 4.9 and 4.10 present the summary of the effects of stirring on the stability of the BGNF- and BGNS-stabilized emulsions. The trend observed showed an increase in stability and decrease in droplet sizes with increasing stirring rate.

Table 4.9 Summary of the influence of stirring on the stability of BGNF- and BGNS-stabilized emulsion

	9000 rpm		12000 rpm		15000 rpm		18000 rpm		21000 rpm	
	Stability (%)									
DAYS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS
0	100	100	100	100	100	100	100	100	100	100
1	70	8	80	14	95	18	95	23	100	27
2	40	4	65	10	85	13	90	16	90	17
3	30	2	50	6	75	8	80	8	85	8.5
4	20	2	35	4	75	5	80	7	85	7.5
5	10	0	30	2.5	75	4	80	7	80	7

Table 4.10 Summary of the influence of stirring on the droplet size of BGNF- and BGNS-stabilized emulsion

	9000 rpm		12000rpm		15000rpm		18000rpm		21000rpm	
	Emulsion droplet size (µm)									
DAYS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS
0	0.015	0.02	8.00E-03	0.01	6.00E-03	8.00E-03	6.00E-03	8.00E-03	6.00E-03	8.00E-03
1	0.015	0.02	8.00E-03	0.01	6.00E-03	8.00E-03	6.00E-03	8.00E-03	6.00E-03	8.00E-03
2	0.018	0.022	9.00E-03	0.011	7.00E-03	8.00E-03	6.90E-03	9.00E-03	6.00E-03	9.00E-03
3	0.02	0.025	0.01	0.013	7.00E-03	9.00E-03	7.00E-03	0.01	6.00E-03	9.00E-03
4	0.022	0.03	0.01	0.015	8.00E-03	0.01	8.00E-03	0.01	6.90E-03	9.00E-03
5	0.024	0.034	0.011	0.017	8.00E-03	0.012	8.00E-03	9.90E-03	7.00E-03	8.00E-03

4.6.1a Stirring effects on BGNF-stabilized emulsions

Storage time and stirring had significant ($p < 0.05$) effect on the MR of BGNF-stabilised emulsions (Table 4.11). The storage time significantly ($p < 0.05$) increased the MR but showed no significant ($p > 0.05$) effect on droplet size. The interaction between storage time and stirring had a significant ($p < 0.05$) effect on MR. This indicates that the influence of storage time on MR differs within the homogenization speed range. Increase in stirring significantly ($p < 0.05$) decreased MR and not the droplet size.

Figure 4.24 and 4.25 show the representative Turbiscan® profile for the effect of homogenization speed on emulsion stability. The homogenization speed had a strong influence on the ability of the emulsion to withstand early creaming. As the homogenization speed increased from 9000 - 21000 rpm, the MR decreased from 0.3195 ± 0.0036 mm/min to 0.009 ± 0.0035 mm/min on day 1. The same trend was observed in relative stability over storage duration period of 5 days (Figure 4.26). Relying on the theoretical framework on the ability of the highest homogenization speed to produce the

Table 4.11 Tests of Between-Subjects effects for migrations rate of BGNF emulsions prepared by varying homogenization speed

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.024[b]	24	0.001	29.569	0.000
Intercept	0.058	1	0.058	1745.610	0.000
Day	0.007	4	0.002	52.468	0.000
Homogenization speed	0.014	4	0.004	107.461	0.000
Day * Homogenization speed	0.002	16	0.000	4.372	0.001
Error	0.001	25	3.316E-005		
Total	0.082	50			
Corrected Total	0.024	49			

[b.] R Squared = 0.966 (Adjusted R Squared = 0.933)

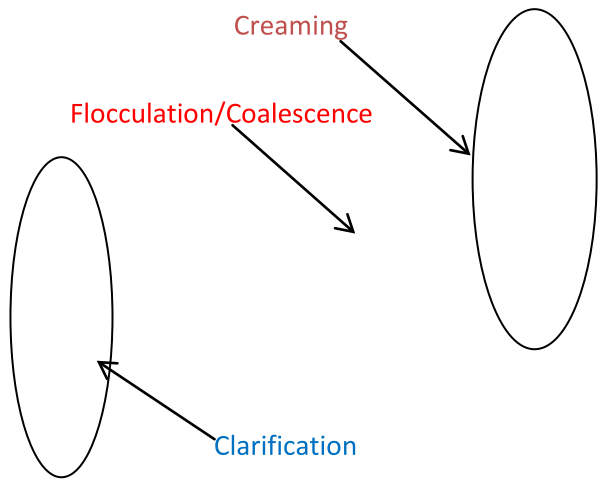


Figure 4.24 Effect of stirring at 12000 rpm on BGNF Stabilized emulsion on day 1

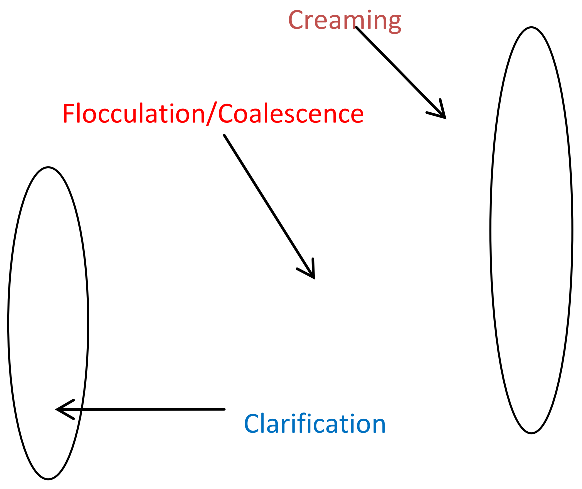


Figure 4.25 Effect of stirring at 12000 rpm on BGNF Stabilized emulsion on day 5

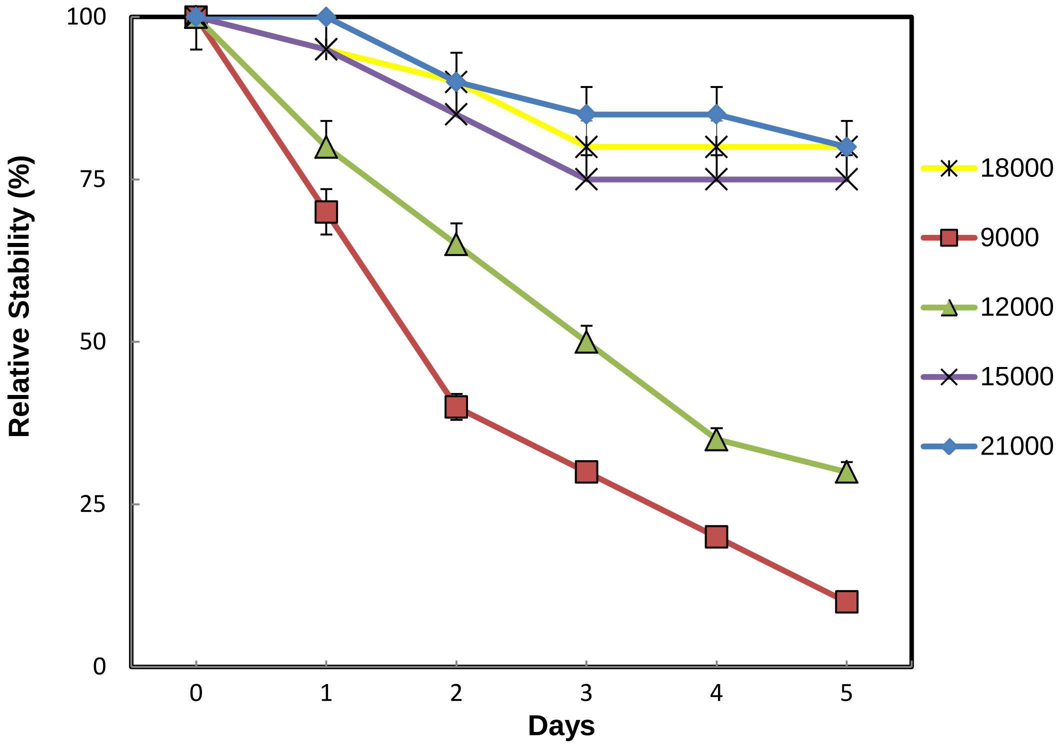


Figure 4.26 The relative stability of BGNF-stabilized emulsions prepared under varying homogenization

best dispersal initially, the immediate stability (just after homogenization) of the emulsion prepared at 21000 rpm (the highest practically available speed in the current study setting) was taken to be 100%. The immediate and subsequent stability of the other emulsions were measured relative thereto. The data obtained from the evaluation of stirring effect on stability of BGNF emulsion revealed that increase in homogenization speed enhances emulsion stability. Similar result was reported by Tornberg and Hermansson (2006) in their work to study the effect of processing on the functional characterization of protein stabilized emulsions. The report showed that by increasing emulsifying intensity, better emulsions were obtained when characterized by the creaming method.

However, the relative stability at the end of each storage period showed decreased trend with migration rate of 0.0172 ± 0.0091 mm/min on day 1 to 0.0452 ± 0.0261 mm/min on day 5. The MR values measured after each storage duration were significantly ($p < 0.05$) different from each other, although the relative stability for day 5 was slightly lower than that for day 4 (Figure 4.26).

The use of droplet size can reveal more clearly the effectiveness of the various homogenization speeds applied in homogenizing the emulsions. The growth of droplet size of each of the emulsions prepared under different homogenization speed is shown in Figure 4.27 and 4.28. There was an inverse relationship in flour emulsion droplet size with increase in homogenization speed. The droplet size decreased from 0.0145 ± 0.0070 µm at homogenization speed of 9000 rpm to 0.0060 ± 0.0001 µm at homogenization speed of 21000 rpm on day 1. Similar trend was observed for each homogenization speed evaluation at the end of the storage duration periods. Droplet size on day 5 was highest 0.0239 ± 0.0006 µm at homogenization speed of 9000 rpm and with the least droplet size of 0.0071 ± 0.0006 µm at homogenization speed 21000 rpm on day 5. However, the measured droplet size of the flour emulsion over the storage period appeared to be homogenous within the cluster of droplet size. Hence, the effect of homogenization speed on flour emulsion appears not to be significant ($p > 0.05$) except for that observed on day 1 (Figure 4.28). The effect of homogenization speed of 21000 rpm to 12000 rpm on droplet size was not significantly different ($p > 0.05$), while homogenization speeds of 21000 rpm and 9000 rpm were significantly different ($p <0.05$).

The use of high speed in homogenizing immiscible liquids is known to help reduce the droplet size and the creaming/sedimentation rate of the emulsions so produced this was a study carried out by Floury et al. (2000) using high-pressure homogenizer which showed that Droplet size reduced with increasing pressure. This usually leads to improved shelf life of the emulsion (Floury et al., 2003).

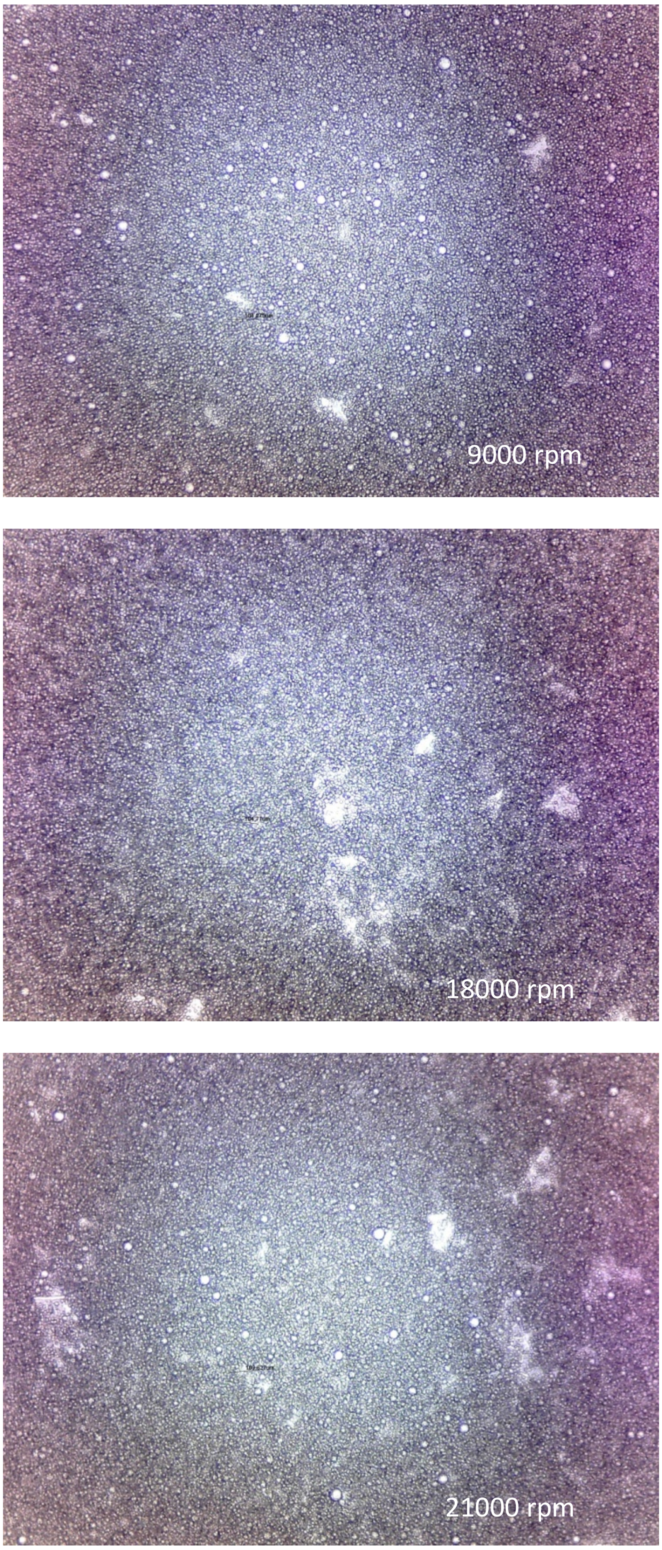


Figure 4.27 Effect of homogenization speed of 9000, 18000 and 21000 rpm on oil droplet size of BGNF-stabilized emulsion

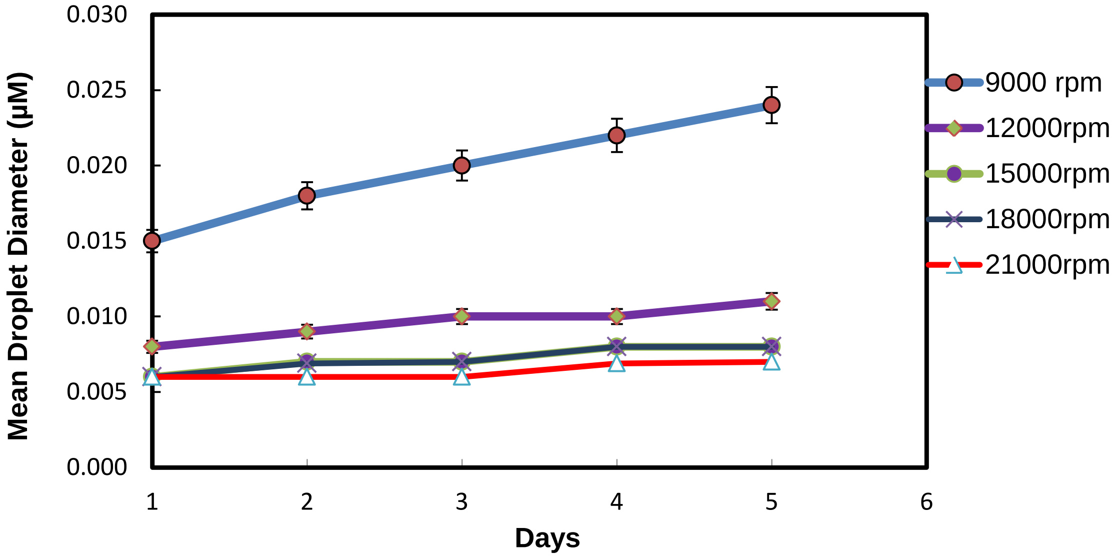


Figure 4.28 The growth of the mean droplet diameter of BGNF-stabilized emulsions prepared under varying homogenization speed

Apart from the reduction in the mean droplet diameter and creaming/sedimentation rate of emulsions, high homogenization are required to evenly break fatty globules and to deflocculates clusters of poorly formed droplets. In addition, high-pressure homogenization leads to increased surface activity of the emulsifier helping to improve their emulsifying capacity (Floury et al., 2003) and this was also observed in the current study, where the initial mean droplet sizes in the two emulsions vary with increased homogenization speed.

The day 5 stability and mean droplet sizes of the emulsions are seen to be better in emulsions with higher homogenization speed (21000 rpm). Emulsion produced using high homogenization speed will have longer shelf life than those prepared with lower Speed. High homogenization subjects the oil and water mixture to intense turbulence and shear flow. In a high-pressure homogenizer, the oil and water mixture is subjected to intense turbulent and shear flow fields. According to Floury et al. (2000), turbulence is the predominant mechanism through which emulsification occurs while laminar shear and cavitations also play significant roles. The dispersed phase is usually broken up into small droplets by turbulence occasioned by high-pressured homogenization.

It should be pointed out that the linear relationship between high homogenization speed and emulsion stability is not infinite. It has been shown that certain emulsifiers especially proteins, can lose their emulsifying property at very high turbulence. This can be attributed to pressure-dependent disruption of the tertiary and quaternary structure of the proteins leading to denaturation (Dumay et al., 1996). Galazka et al. (1999) reported the loss of emulsifying capacity of β-lactoglobulin as a result of high-pressure treatment. Similar effects have been shown in whey protein-stabilized emulsions (Galazka et al., 1995).

The result of the current study showed that BGNF influenced both the long-term (up to day 5) stability and mean droplet diameters of the emulsions so prepared irrespective of the stirring rate as well as the initial droplet size and stability of the emulsions. Hence, it is in agreement with the hypothesis that the stirring rate increase BGNF emulsion stability significantly ($p < 0.05$).

4.6.1b Stirring effects on BGNS-stabilized emulsions

Storage time and homogenization speed had significant ($p < 0.05$) effect on the MR and droplet size of BGNS-stabilised emulsions (Figures 4.29 and 4.30). The storage time significantly ($p < 0.05$) increased the MR and droplet size. The interaction between storage time and homogenization speed had a significant ($p < 0.05$) effect on MR and the droplet size (Tables 4.12 and 4.13).

Table 4.12 Tests of Between-Subjects effects for migrations rate of BGNS emulsions prepared by varying homogenization speed

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.044[b]	24	0.002	9.610	0.000
Intercept	0.106	1	0.106	562.064	0.000
Day	0.017	4	0.004	22.023	0.000
Homogenization speed	0.020	4	0.005	26.925	0.000
Day * Homogenization speed	0.007	16	0.000	2.178	0.039
Error	0.005	25	0.000		
Total	0.155	50			
Corrected Total	0.048	49			

[b.] R Squared = 0.902 (Adjusted R Squared = .808)

Table 4.13 Tests of Between-Subjects effects for droplet sizes of BGNS emulsions prepared by varying homogenization speed

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.002[a]	24	9.862E-005	129.695	0.000
Intercept	0.009	1	0.009	12154.867	0.000
Day	0.000	4	5.040E-005	66.277	0.000
Homogenization speed	0.002	4	0.001	662.673	0.000
Day * Homogenization speed	0.000	16	9.357E-006	12.306	0.000
Error	1.901E-005	25	7.604E-007		
Total	0.012	50			
Corrected Total	0.002	49			

[b]R Squared = 0.992 (Adjusted R Squared = .984)

This indicates that the influence of storage time differ within the homogenization speed. Increase in homogenization speed decreased MR and droplet size.

Using migration rate as a measure of creaming/sedimentation, the relative stability of the BGNS-stabilized emulsions prepared under varying homogenization rate is presented in Figure 4.31. The homogenization rate was varied by stirring at 9000, 12000, 15000, 18000 and 21000 rotations per minute (rpm). Relying on the theoretical framework on the ability of the highest homogenization speed to produce the best dispersal initially, the immediate stability (just after homogenization) of the emulsion prepared at 21000 rpm (the highest practically available speed in the current study setting) was taken to be 100%. The immediate and subsequent stability of the other emulsions were measured relative thereto. Figure 4.29 and 4.30 shows the Turbiscan® destabilisation kinetics profiles for the effects of homogenization speed on emulsion for day 1 and day 5, others are in appendix 1.

The relative stability of the starch emulsion showed a direct relationship with increase in homogenization speed. The least relative stability for all emulsion from day 1 to day 5 storage duration were observed at homogenization speed 21000 rpm, while the maximum mean relative stability were recorded at homogenization speed 9000 rpm. Thus, the starch emulsion on day 1 appeared to be most stable, while other days were the least. However, homogenization speed of 21000 rpm resulted in nuisance as the stirring noise level was high, thus homogenization speed of 15000 rpm was held acceptable in respect of the laboratory scale. Industrial designs may therefore consider accommodating homogenization speed greater than 21000 rpm. There was significant difference ($p < 0.05$) between relative stability over the storage duration period, although, MR value of emulsion on day 2 and day 3, were not significantly different ($p > 0.05$). Also the relative stability value measured on day 4 and day 5 were not significantly different ($p > 0.05$).

One of the important findings of this study is that the stability and/or mean droplet size vary depending on whether they were stabilized by BGNF or BGNS. It showed that the combination of the speed and the emulsifying capacity of the additive was what made the homogenization effective.

Figure 4.32 shows the growth of the droplet diameter with time while Figure 4.33 provides the graphical representation of the mean droplet diameter of the BGNS-stabilized emulsions prepared under varying stirring rate. The droplet size of the BGNS emulsion observed under varying homogenization speed showed that the highest droplet size of 0.0210 ± 0.0014 µm was obtained from the lowest homogenization speed (9000 rpm) and the lowest droplet value of 0.0082 ± 0.0002 µm was obtained from the homogenization speed of 15000 rpm and 18000 rpm on day 0 while the highest homogenization speed (21000 rpm) gave the droplet size of 0.00825 ± 0.0003 µm.

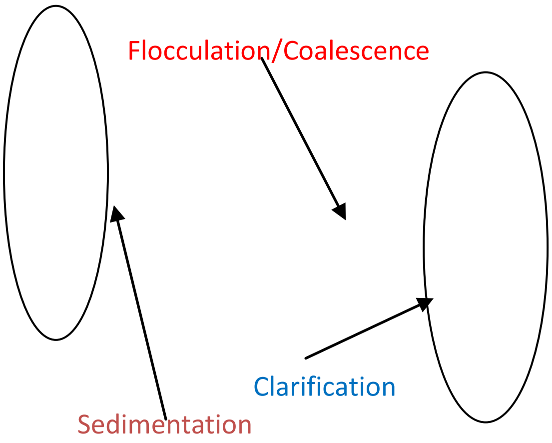


Figure 4.29 Turbiscan destabilization profile showing the effect of stirring at 12000 rpm on BGNS-stabilized emulsion on day 1

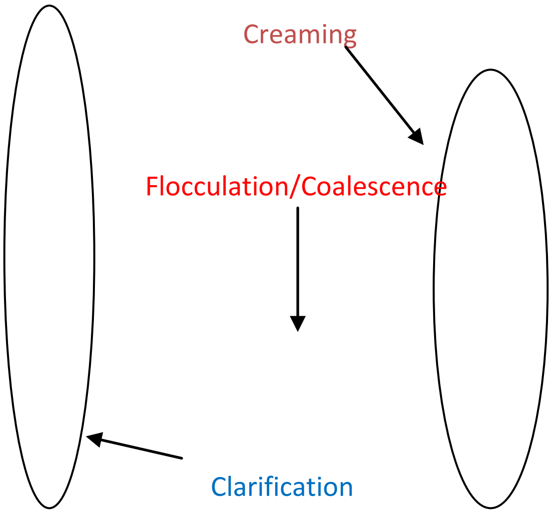


Figure 4.30 Turbiscan destabilization profile showing the effect of stirring at 12000 rpm on BGNS-stabilized emulsion on day 5

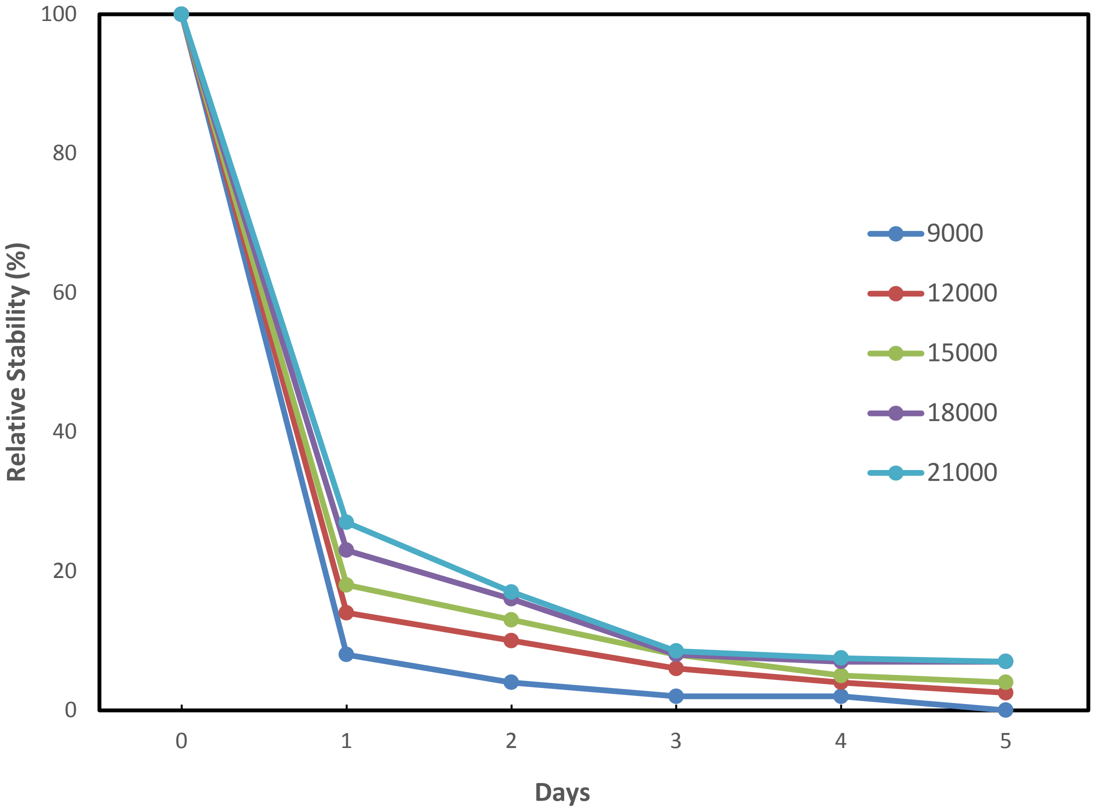


Figure 4.31 The relative stability of BGNS-stabilized emulsions prepared under varying homogenization speed

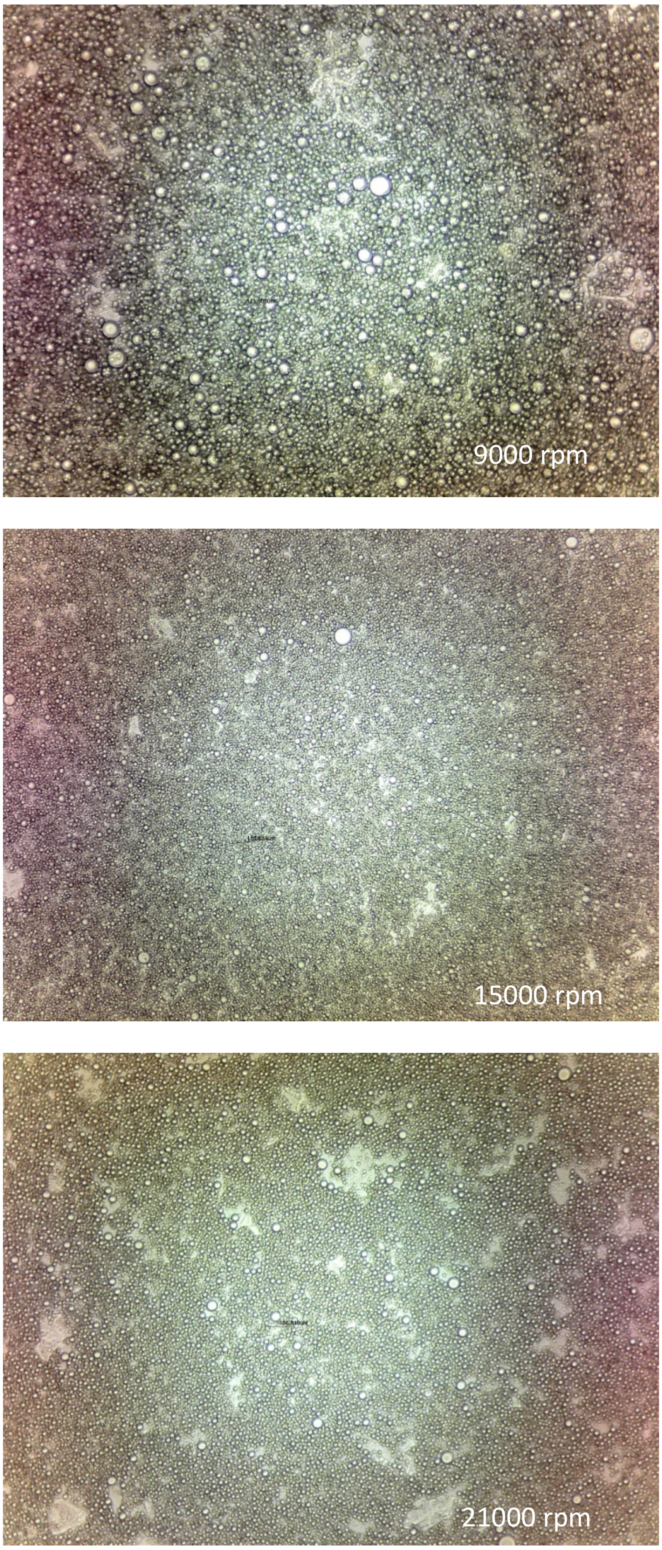


Figure 4.32 Effect of homogenization speed of 9000 rpm, 15000 rpm and 21000 rpm on the oil droplet size of BGNS-stabilized emulsion

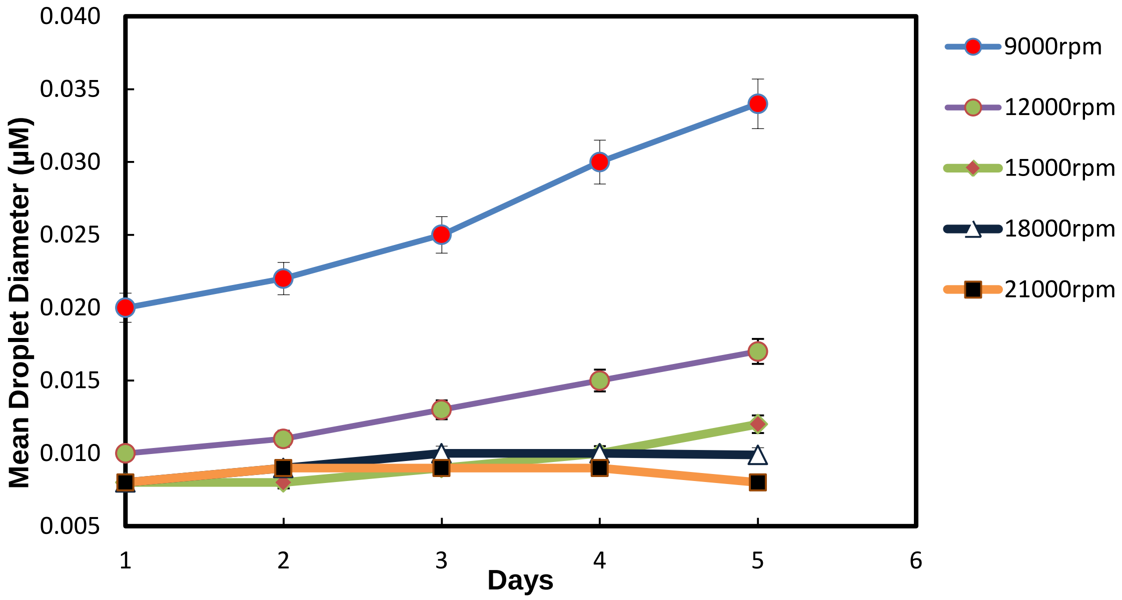


Figure 4.33 The growth of the mean droplet diameter of BGNS-stabilized emulsions prepared under varying homogenization speed

On the day 2 of storage till day 5, the homogenization speed of 21000 rpm was observed to have the lowest droplet size. Also the mean droplet size obtained showed that there was significant ($p < 0.05$) decrease in the droplet size with increasing homogenization speed, where the mean droplet size of 0.0258 ± 0.0052 µm was obtained for the lowest homogenization speed (9000 rpm) and the highest Homogenization speed (21000 rpm) have the lowest mean droplet size of 0.0089 ± 0.0069 µm. More so, the Homogenization speed of 21000 rpm, 18000 rpm and 15000 rpm were not significantly different ($p > 0.05$) from one another but the homogenization speed of 15000 rpm, 12000 rpm and 9000 rpm showed a significant different ($p < 0.05$).

It was also observed that the mean droplet obtained for each storage days showed a significant ($p < 0.05$) increase with storage time, where day 0 had the lowest mean droplet size of 0.0112 ± 0.0052 µm and day 5 showed the mean droplet size of 0.0101 ± 0.0096 µm. There was no significant difference ($p > 0.05$) between the mean droplet size of day 1 and day 2 but the mean droplet size of day 3 to day 5 increased significantly ($p < 0.05$). The trend of the stability in BGNS-stabilized emulsion is generally similar to the one observed in BGNF-stabilized one. There was however, a marginally higher degree of stability with time in BGNF-stabilized emulsions as compared to BGNS - stabilized one. Similar observation was true for the mean droplet size. The initial mean droplet size of the emulsions stabilized with BGNS was higher than the droplet sizes of BGNF-stabilized one. As noted earlier, this further suggests that BGNF might be more effective as an emulsifier than BGNS. In both BGNF and BGNS-stabilized emulsions, emulsion stability increased with increased homogenization speed. The higher the homogenization speed, the smaller the initial droplet size.

The result of the current study shows that BGNF and BGNS not only influenced the long-term (up to day 5) stability and mean droplet diameters of the emulsions so prepared irrespective of the homogenization speed, they also had significant effects on the initial droplet size and stability of the emulsions. This agrees with the initial hypothesis of the study that BGNF and BGNS are capable of stabilizing emulsions.

4.6.2 Effects of pH on BGNF-stabilized emulsions

Tables 4.14 and 4.15 present the summary of the influence of pH on the stability and droplet sizes of the emulsions stabilized by BGNF and BGNS

Table 4.14 Summary of the influence of pH on the stability of BGNF- and BGNS-stabilized emulsion

	pH 2		pH 4		pH 6		pH 8		pH 10	
	Relative stability (%)									
DAYS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS
0	100	100	100	100	100	100	100	100	100	100
1	94	92	96	95	99	98	97	95	97	94
2	91	84	93	88	97	94	95	91	95.3	90.7
3	88	80	91	84	95	91	93	88	92.5	87
4	87	76	90	81	95	90	92	86	92	87
5	84	71	88	74	94.5	90	92	85	90.69	84

Table 4.15 Summary of the influence of pH on the droplet size on the BGNF- and BGNS-stabilized emulsions

	PH 2		PH 4		PH6		PH 8		PH10	
	Droplet size (µm)									
DAYS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS
0	6.00E-03	0.01	7.00E-03	0.01	6.00E-03	0.01	8.00E-03	0.01	8.00E-03	0.01
1	6.00E-03	0.01	7.00E-03	0.01	6.00E-03	0.01	8.00E-03	0.01	8.00E-03	0.01
2	0.018	0.02	9.00E-03	0.017	7.00E-03	0.012	8.00E-03	0.015	9.00E-03	0.017
3	0.02	0.034	0.01	0.032	7.00E-03	0.015	0.011	0.023	0.01	0.026
4	0.024	0.04	0.011	0.035	8.00E-03	0.018	0.011	0.027	0.011	0.031
5	0.025	0.05	0.012	0.044	8.00E-03	0.02	0.012	0.032	0.012	0.038

4.6.2a Effects of pH on BGNF-stabilized emulsions

Storage time and pH had significant ($p < 0.05$) effect on the MR and droplet size of BGNF-stabilised emulsions. The storage time significantly ($p < 0.05$) increased the MR and droplet size. The interaction between storage time and pH had a significant ($p < 0.05$) effect on MR and the droplet size. This indicates that the influence of storage time differed within the pH range. Increase in pH decreased MR and droplet size (Table 4.16 and 4.17).

Table 4.16 Tests of Between-Subjects effects for migrations rate of BGNF emulsions prepared at varying pH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.014[b]	24	.001	20.776	.000
Intercept	.070	1	.070	2449.012	.000
Day	.008	4	.002	66.974	.000
pH	.006	4	.001	48.877	.000
Day * pH	.001	16	6.313E-005	2.201	.037
Error	.001	25	2.868E-005		
Total	.085	50			
Corrected Total	.015	49			

[b] R Squared = .952 (Adjusted R Squared = .906)

Table 4.17 Tests of Between-Subjects effects for droplet sizes of BGNF emulsions prepared at varying pH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.001[b]	24	5.178E-005	183.085	0.000
Intercept	0.006	1	0.006	21988.526	0.000
Day	0.000	4	7.074E-005	250.149	0.000
pH	0.001	4	0.000	637.722	0.000
Day * pH	0.000	16	1.489E-005	52.660	0.000
Error	7.070E-006	25	2.828E-007		
Total	0.007	50			
Corrected Total	0.001	49			

[b] R Squared = 0.994 (Adjusted R Squared = .989)

The observed trend in the emulsion MR when the pH was varied from pH 2 - pH 10 per day showed that the emulsions prepared with pH 2 has the highest MR of 0.2645 ± 0.0048 mm/min on day 1 and MR of 0.0789 ± 0.00551 mm/min on day 5 for the emulsions in the acidic medium (pH 2, pH4 and pH6). Emulsions at pH 10 showed the highest MR of 0.0184 ± 0.0032 mm/min on day 1 and MR of 0.0591 ± 0.0087 mm/min on day 5 for the emulsion in the alkaline medium (pH 8 and pH10). The MR observed at pH 6 showed the lowest value of 0.0076 ± 0.0022 mm/min on day 1 and 0.0280 ± 0.0005 mm/min on day 5. The mean MR of 0.0223 ± 0.0940 mm/min at pH 6 was the lowest which suggests that the emulsion is most stable at pH 6. More so, the mean MR of pH 6 decreased significantly ($p < 0.05$) from the mean MR obtained for other pH values except from the mean MR of pH 10 and pH 4 which were not significantly different ($p > 0.05$) from one another. Neumann and Paczynska-Lahme (1996) reported a decreased stability of crude oil-in-water emulsion with increased pH and decreased electrolyte concentration where the emulsion was found to be least stable at pH 10.5.

There was significant ($p < 0.05$) increase in the mean MR for different pH with storage time. The least MR of 0.0184 ± 0.0073 mm/min was obtained on day 1 and it was significantly ($p < 0.05$) different from the mean MR observed throughout the storage duration except from those observed between day 3 and day 4, which are not significantly different ($p > 0.05$). This implies that the BGNF emulsion is stable at pH 6 (Figures 4.34 and 4.35), showed the emulsion at pH 6 as the most stable. Emulsion stability improved with increased pH till pH 7, beyond which stability decreased. This linearity showed that the more acidic the emulsion medium the more unstable they were. Thus, both acidity and alkalinity reduces the emulsion stability (Figures 4.36 and 4.37). (See more Turbiscan® profiles showing effect of pH on emulsion stability with time in Appendix 2).

The effect of varied pH on the droplet size of the flour emulsion was not strictly definite and this varied over the storage duration (Figure 4.38). Droplet size observed at pH 2 on day 1 was the lowest but its mean droplet size was not the lowest while the droplet size measured at pH 6 on day 2 to day 5 were the lowest. The droplet size increased with increase in storage duration. The overall mean droplet size observed on day 1 (0.0069 ± 0.0100 µm) was the least while day 5 (0.0136 ±0 .0057 µm) was the highest. However, the mean droplet size observed after each storage duration increased significantly ($p < 0.05$). The variability of droplet value observed from BGNF-stabilized emulsion after storage duration of day 2 to day 4 was comparable. Also, the mean droplet size observed for pH 4 to pH 10 were not significantly different from one another ($p > 0.05$), although there were significant difference ($p < 0.05$) between emulsion droplet size at pH adjusted to pH 2 and pH 4.

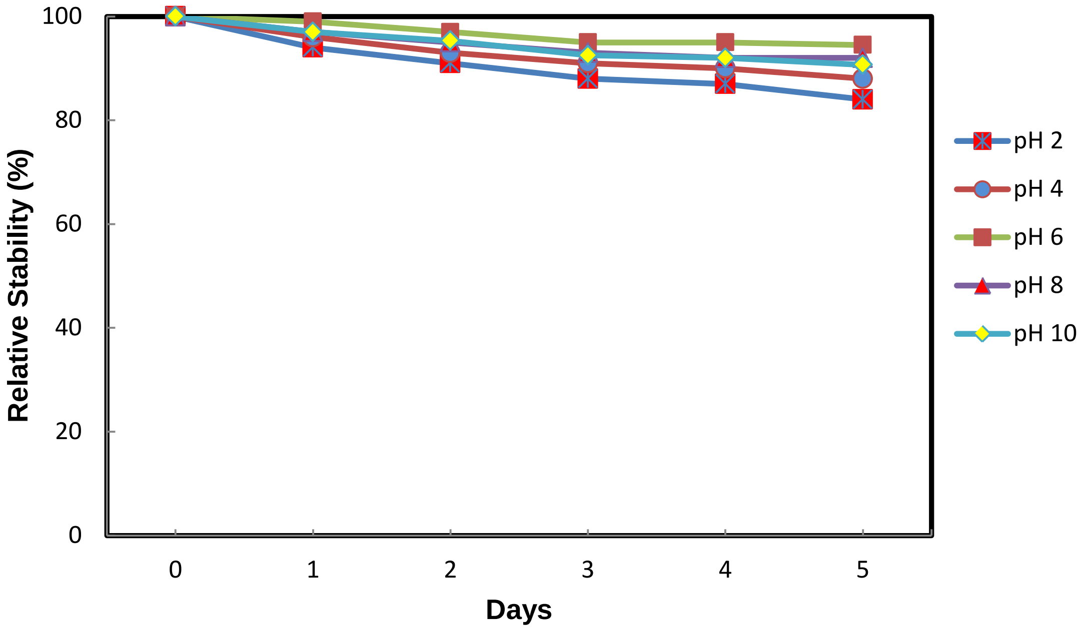


Figure 4.34 The influence of pH on the relative stability of BGNF-stabilized emulsions

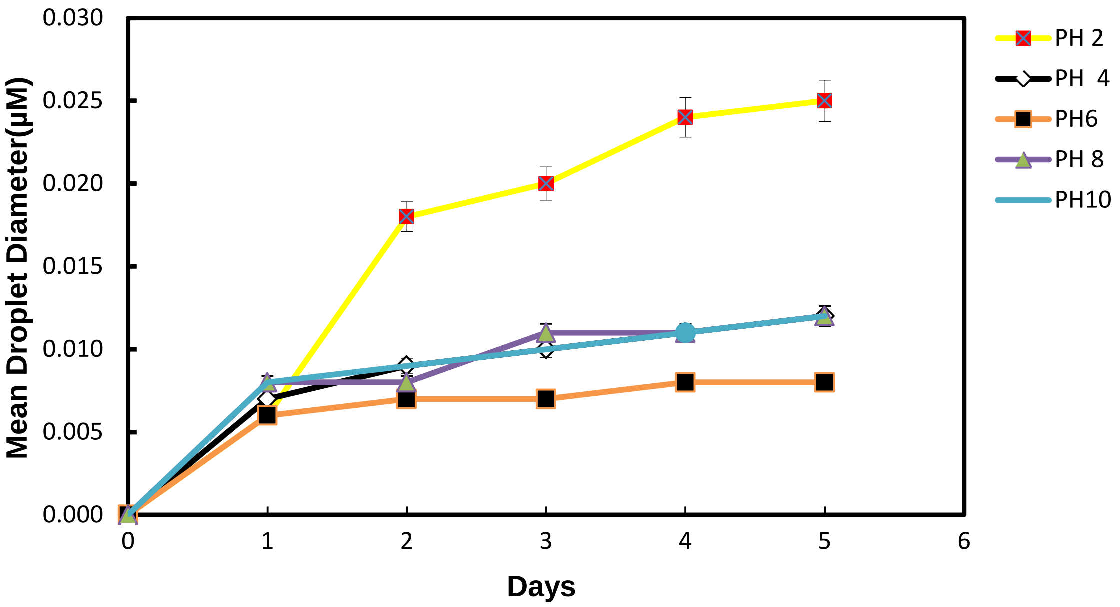


Figure 4.35 The influence of pH on the mean droplet diameter of BGNF-stabilized emulsions

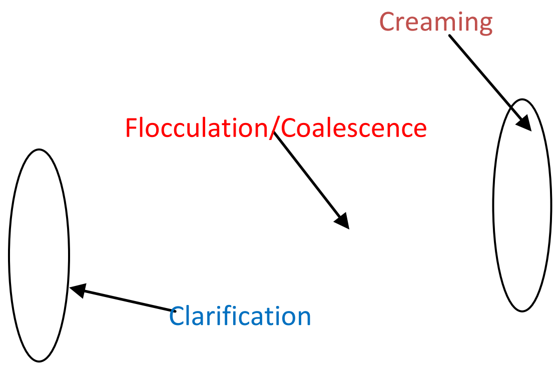


Figure 4.36 Effect of pH on BGNF Stabilized emulsion on day 1

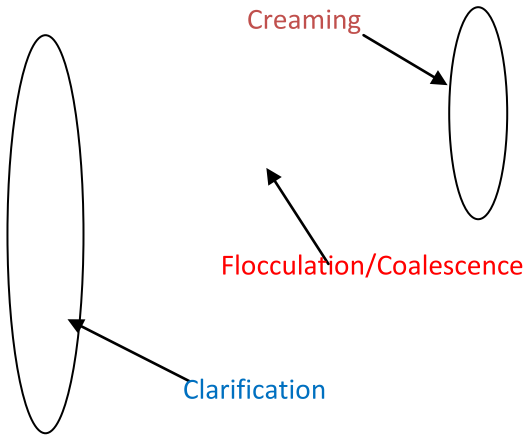


Figure 4.37 Effect of pH on BGNF Stabilized emulsion on day 5

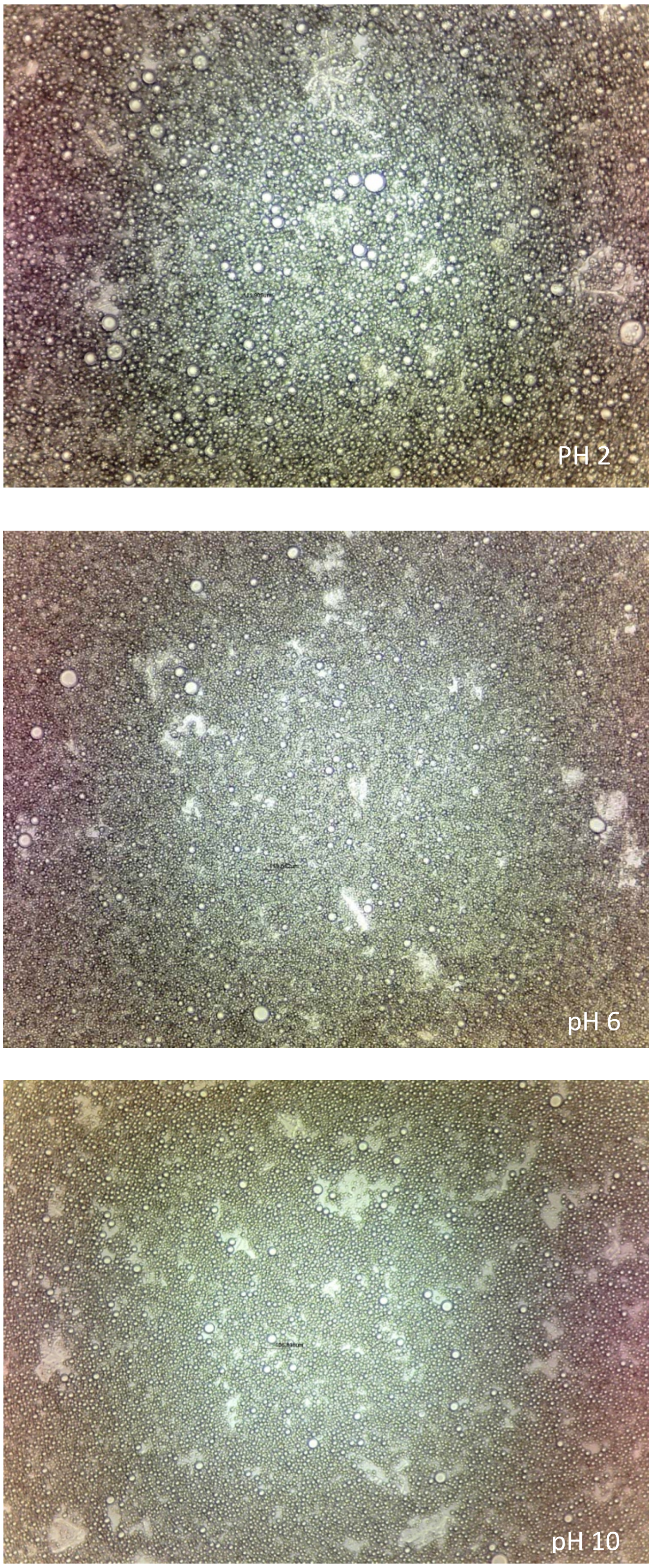


Figure 4.38 Effects of pH 2, pH 6 and pH10 on the oil droplet size of BGNF stabilized emulsion

With the BGNF-stabilized emulsions, migration rates and the mean droplet diameters immediately after homogenization varied. This implies that even the effectiveness of the homogenization was influenced by pH.

Thus, from the result obtained from both MR and droplet size, emulsion adjusted to pH less than pH 6 and or adjusted to pH greater than pH 7 may not encourage stability. The result also showed that storage of BGNF emulsion with pH over time may have a counter-productive effect on emulsion stability. The knowledge of the effect of pH on the stability of food, pharmaceutical and industrial emulsions is important in order to predict product shelf life especially when the emulsions contain acidic or alkaline additives. Yang et al. (2007) reported similar findings in which the stability of the emulsion increased with increasing pH up to 9 beyond which stability decreases. In addition, the effect of pH on BGNF-stabilized emulsion as seen earlier (section 4.5.1) showed that protein was not solely responsible for such emulsification. This is because, proteins, being amino acids, are more soluble at higher pH and are thus expected to have stronger emulsifying capacity at alkaline pH. In the current study, emulsion stability increased only to between pH 7 and 8. This suggests that the emulsifying strength of BGNF may not be solely due to the protein in it.

4.6.2b Effects of pH on BGNS-stabilized emulsions

Storage time and pH had significant ($p < 0.05$) effect on the MR and droplet size of BGNS-stabilised emulsions. The storage time significantly ($p < 0.05$) increased the MR and droplet size. The interaction between storage time and pH had a significant ($p < 0.05$) effect on MR and the droplet size (Table 4.18 and 4.19). This indicates that the influence of storage time differed within the pH range. Increase in pH decreased MR and droplet size (Figures 4.39 and 4.40) with the emulsion at pH 6 having the least MR in BGNS-stabilized emulsion, which is similar to the observation in BGNF-stabilized emulsions. However, the effect of pH 8 and 10 did not differ significantly ($p < 0.05$). Emulsion stability improves with increasing pH till pH 7, beyond which stability decreases. Thus, both acidity and alkalinity reduces the emulsion stability. The more acidic the emulsion medium the less stable they were. The migrations rates and the mean droplet diameters measured immediately after homogenization varied in the BGNS-stabilized emulsions.

The least MR was on day 1 with mean MR of 0.0149 ± 0.0041 mm/min for pH 6 and maximum MR of 0.3655 ± 0.0029 mm/min at pH 2. There was however a progressive increase in MR at pH 6 with the highest on day 5. Thus the stability of starch emulsions were maximum at pH 6 on day 1 and on day 5. The Turbiscan® profile showing the effects of pH on emulsion stability is shown in Figure 4.41 and 4.42. The emulsion MR over the storage duration were however significantly ($p < 0.05$) different.

Table 4.18 Tests of Between-Subjects effects for migrations rate of BGNS emulsions prepared at varying pH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.052[b]	24	0.002	59.995	0.000
Intercept	0.202	1	0.202	5551.699	0.000
Day	0.029	4	0.007	195.809	0.000
pH	0.019	4	0.005	127.654	0.000
Day * pH	0.005	16	0.000	9.127	0.000
Error	0.001	25	3.641E-005		
Total	0.255	50			
Corrected Total	0.053	49			

[b] R Squared = .983 (Adjusted R Squared = .967)

Table 4.19 Tests of Between-Subjects effects for droplet sizes of BGNS emulsions prepared at varying pH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.006[b]	24	0.000	139.083	0.000
Intercept	0.029	1	0.029	14811.035	0.000
Day	0.004	4	0.001	567.017	0.000
pH	0.001	4	0.000	183.744	0.000
Day *pH	0.001	16	4.068E-005	20.934	0.000
Error	4.858E-005	25	1.943E-006		
Total	0.035	50			
Corrected Total	0.007	49			

[b] R Squared = .993 (Adjusted R Squared = 0.985)

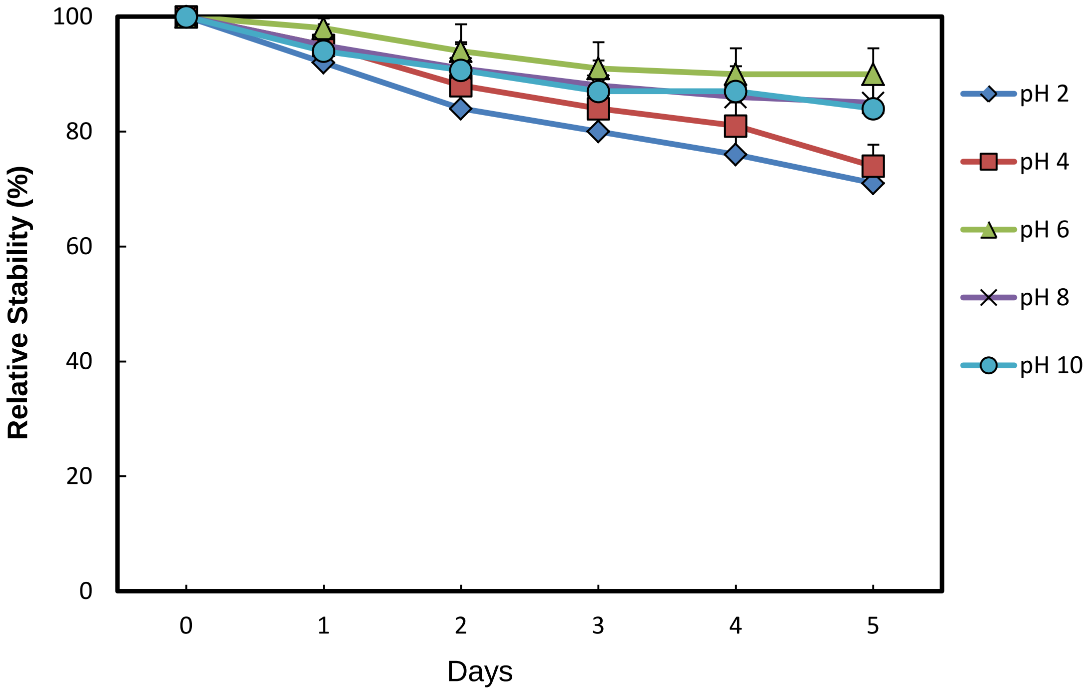


Figure 4.39 The influence of pH on the relative stability of BGNS-stabilized emulsions

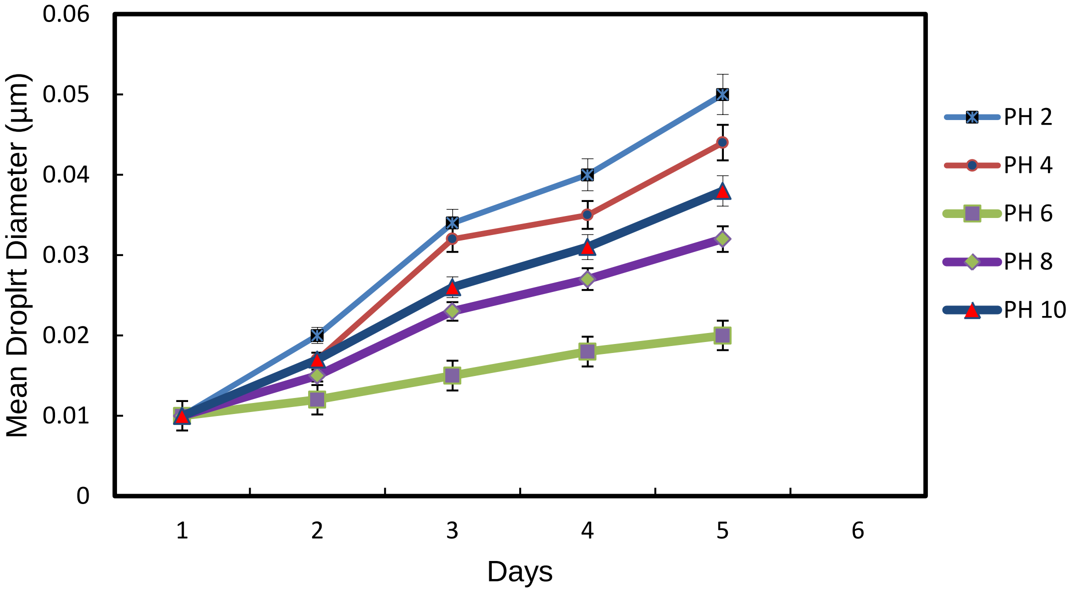


Figure 4.40 The influence of pH on the mean droplet diameter of BGNS-stabilized emulsions

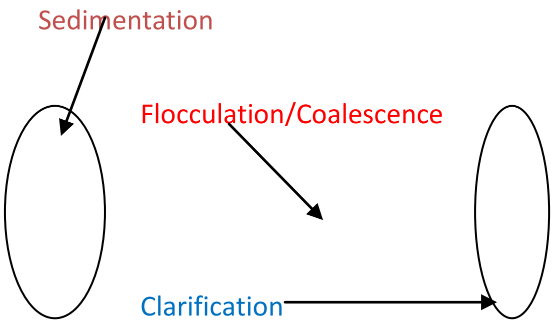


Figure 4.41 Effect of pH on BGNS Stabilized emulsion on day 1

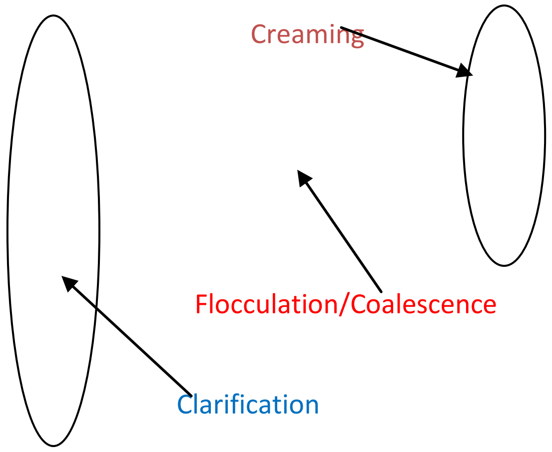


Figure 4.42 Effect of pH on BGNS Stabilized emulsion on day 5

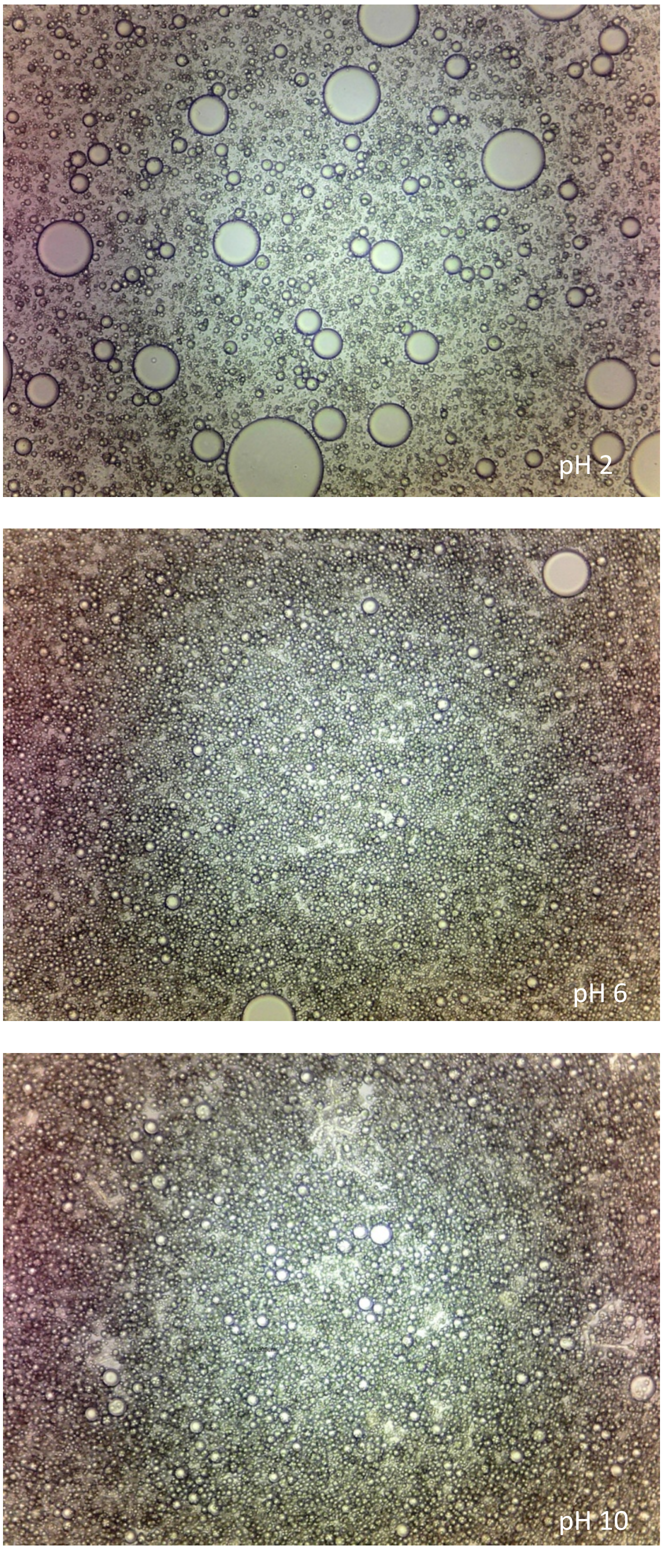


Figure 4.43 Effects of pH 2, pH 6 and pH 10 on oil droplet of BGNS stabilized emulsion

from one another. Change in pH did not have significant effect on the droplet size on day 1 except at pH 2, which showed the least droplet size of 0.0099 ± 0.0063 µm The trend observed from day 2 to day 5 upon storage showed a definite pattern for droplet size value at pH 6 as the least. Hence, apart from pH 2 on day 1 the critical cluster droplet value was observed at pH 6. On the overall, the mean droplet value observed between the storage duration clusters increased with storage period. Thus, the stability of starch emulsion decreased over time, with droplet value measured between the storage periods being significantly different ($p < 0.05$) from one another. Also the mean droplet size values of the effects of pH were significantly different ($p < 0.05$) from pH 2 to pH 10 with pH 6 having the least mean droplet size (Figure 4.43). The pH effect was more profound on BGNS-stabilized emulsions where up to 30% reduction in stability was observed by day 5 (compared to 16% in BGNF-stabilized emulsions over the same period of time).

Generally the stability observed in BGNS-stabilized emulsions is lower to that observed in the corresponding BGNF-stabilized ones. Comparatively, the mean droplet diameters of the BGNS-stabilized emulsions were twice those of their corresponding BGNF-stabilized emulsion. This suggested that BGNF had the higher emulsion-stabilizing capacity. Proteins are more soluble in alkaline media (Cacioppo and Pusey, 1991). Thus, it is expected that protein-stabilized emulsions will be more stable at alkaline pH. The findings in this study suggested that the emulsion stability property observed in BGNF may not be solely due to its protein content but a mixture of constituents similar to the findings of Nakamura et al., (2004) on soy protein/polysaccharide mixture. The current findings thus agree with the hypothesis of this study that BGN has the capacity to serve as emulsifying agent in food products, in addition to its nutritional property.

4.6.3 Effects of NaCl on the emulsions

Tables 4.20 and 4.21 show the summary of the effects of varying concentration of the NaCl on the stability and droplet size of BGNF-and BGNS-stabilized emulsions.

Table 4.20 Summary of the influence of stirring on the stability of BGNF- and BGNS-stabilized emulsion

	2% NaCl		4% NaCl		6% NaCl		8% NaCl		10% NaCl	
	Relative stability (%)									
DAYS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS
0	100	100	100	100	100	100	100	100	100	100
1	97.77	97	96.67	96	96.77	96.77	96.2	96.2	95.86	95.86
2	92.2	87	91	83	94	87	95	91	95.3	92
3	89	81	88	76	91	83	92	85	92.5	87
4	86	72	85	71	90	79	91	81	92	83
5	83	66	84	67	89	78	90	82	90.69	82

Table 4.21 Summary of the influence of stirring on the droplet size of BGNF- and BGNS-stabilized emulsion

	2 % NaCl		4% NaCl		6% NaCl		8% NaCl		10% NaCl	
	Droplet size (µm)									
DAYS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS
0	6.00E-03	0.01	7.00E-03	0.01	7.00E-03	0.01	8.00E-03	0.01	8.00E-03	0.01
1	6.00E-03	0.01	7.00E-03	0.01	7.00E-03	0.01	8.00E-03	0.01	8.00E-03	0.01
2	7.00E-03	0.01	0.031	0.0275	0.036	0.02	0.022	0.013	0.02	0.01
3	7.00E-03	0.011	0.035	0.039	0.038	0.0315	0.026	0.018	0.023	0.012
4	8.00E-03	0.013	0.036	0.053	0.04	0.045	0.028	0.02	0.024	0.015
5	8.00E-03	0.014	0.036	0.07	0.045	0.05	0.034	0.028	0.026	0.022

4.6.3a Effects of NaCl on BGNF-stabilized emulsions

Storage time and NaCl had significant ($p < 0.05$) effect on the MR and droplet size of BGNF-stabilised emulsions. The storage time significantly ($p < 0.05$) increased the MR and droplet size. The interaction between storage time and NaCl had a significant ($p < 0.05$) effect on MR and the droplet size. This indicates that the influence of storage time differed within the NaCl range (Table 4.22 and 23). Increase in NaCl decreased MR and droplet size. The influence of sodium chloride on the MR of BGNF-stabilized emulsion is presented in Figure 4.44 and the mean droplet diameter is presented in Figure 4.45. The creaming stability of the emulsion increased with increased NaCl concentration. Emulsions containing higher sodium chloride (NaCl) concentration had smaller mean particle diameters (Figure 4.45). Similar observation was made by Guzey et al. (2004) in their study to investigate the effect of NaCl on the stability of O/W emulsion stabilized by

Table 4.22 Tests of Between-Subjects effects for migrations rate of BGNF emulsions prepared at varying NaCl concentration

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.019[b]	24	0.001	17.295	0.000
Intercept	0.101	1	0.101	2182.464	0.000
Day	0.015	4	0.004	80.765	0.000
NaCl	0.002	4	0.001	11.302	0.000
Day * NaCl	0.002	16	0.000	2.926	0.008
Error	0.001	25	4.629E-005		
Total	0.121	50			
Corrected Total	0.020	49			

[b] R Squared = .943 (Adjusted R Squared = 0.889)

Table 4.23 Tests of Between-Subjects effects for droplet sizes of BGNF emulsions prepared at varying NaCl concentration

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.006[b]	24	0.000	150.145	0.000
Intercept	0.031	1	0.031	18811.500	0.000
Day	0.004	4	0.001	616.071	0.000
NaCl	0.001	4	0.000	209.109	0.000
Day * NaCl	0.001	16	3.165E-005	18.922	0.000
Error	4.181E-005	25	1.672E-006		
Total	0.038	50			
Corrected Total	00.006	49			

[b] R Squared = .993 (Adjusted R Squared = .986)

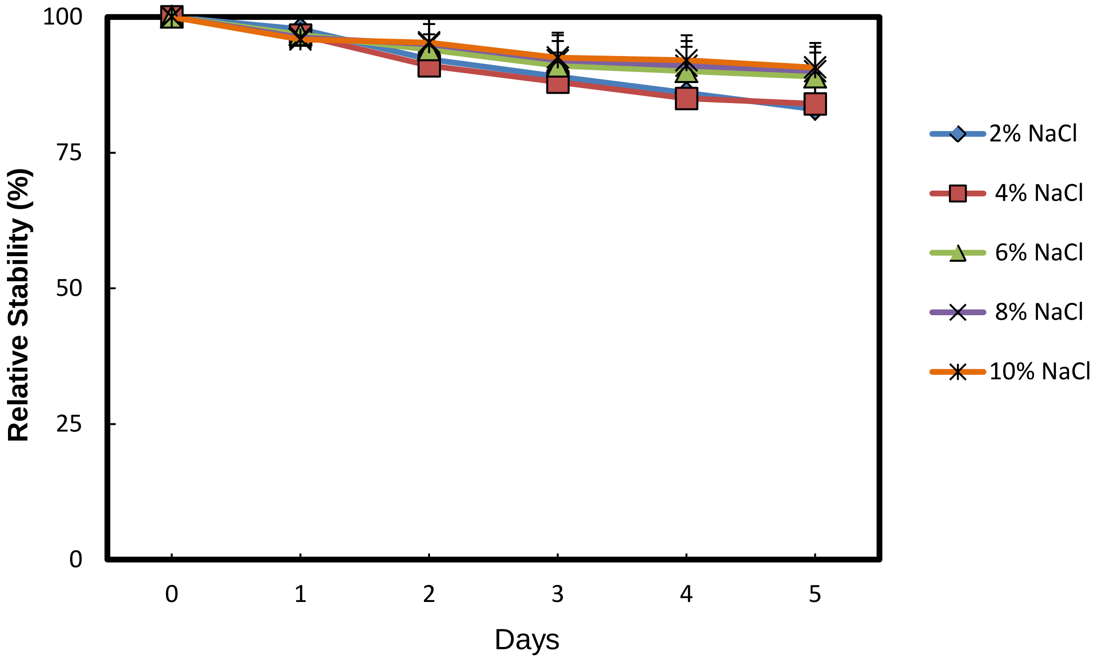


Figure 4.44 The influence of sodium chloride on the relative stability of BGNF-stabilized emulsion

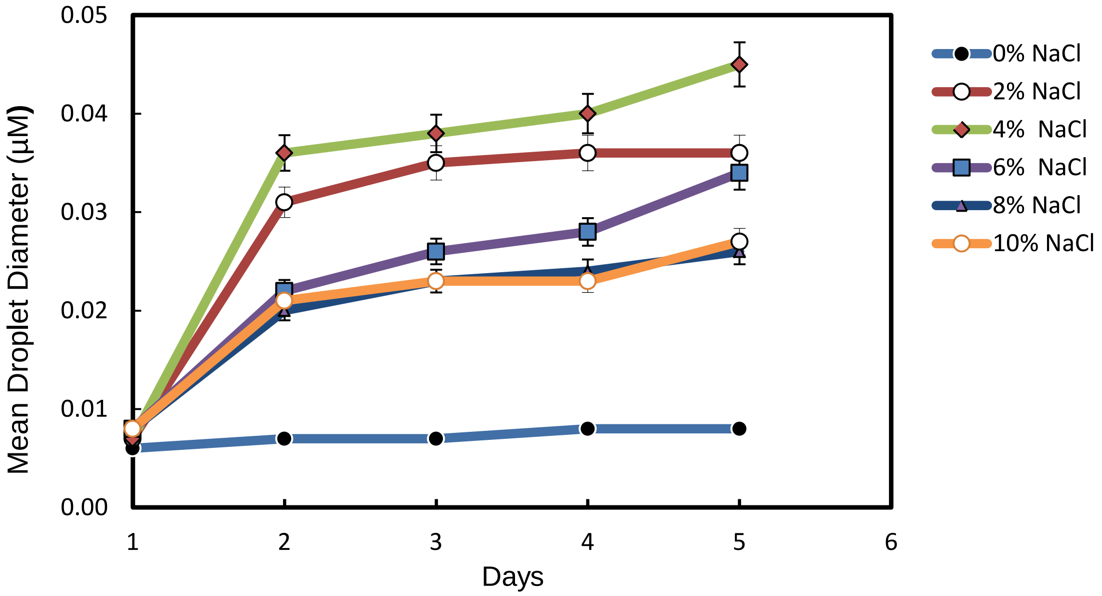


Figure 4.45 The influence of sodium chloride on the mean droplet diameter of BGNF-stabilized emulsions

β-lactoglobulin–pectin membranes. They reported that the emulsions were more stable at higher concentration of NaCl and was attributed to the screened electrostatic interactions between charged regions on the pectin molecules which have influenced the packing of the droplets within the creamed layer formed by the flocculated droplets and this can lead to the decrease in the height of serum layer.

The presence of NaCl did not affect the homogenization efficiency as the immediate droplet sizes are similar on day 1 for all NaCl concentrations (Figure 4.45). This implies that NaCl did not interfere with the BGNF-dependent emulsification process.

Results showed statistically that there was down surge in MR at concentration greater than 4% w/v (i.e. 6% w/v), thereafter a steady increase was observed. On the average, the MR value increased from 0.0158 ± 0.0048 mm/min on day 1 to 0.0634 ± 0.0166 mm/min on day 5. The MR at 10% w/v NaCl salt concentration was the least (0.0381 ± 0.0122 mm/min) while mean MR value at 2% w/v NaCl was the highest (0.0530 ± 0.027 mm/min). The MR of emulsions containing between 2% w/v and 4% w/v NaCl concentrations cluster, were not significantly different ($p > 0.05$) from each other. The MR of the emulsions with NaCl ranged from 6% w/v – 10% w/v NaCl, were also not significantly different ($p > 0.05$) from one another. However, there was significant difference ($p < 0.05$) between the MR of emulsions at 4% w/v and 6% w/v NaCl, and this implies that the threshold of enhanced emulsion stability by the addition of NaCl is at >6% w/v NaCl.

The storage time also had an effect on the MR of BGNF emulsion. The mean MR of the emulsion increased with storage duration between day 1 and day 4, and then decreased slightly on day 5. Although, the within emulsion samples per storage day duration MR were not significantly different ($p < 0.05$) from one another, there were significant differences ($p < 0.05$) between the mean MR value of the emulsions per day for days 1 to day 4, except for day 4 and day 5 which were not significantly different ($p > 0.05$). This implies that the MR increased significantly from day 1 to day 5 (Figure 4.46 and 4.47 other Turbiscan® profiles showing effect of NaCl on emulsion stability are in Appendix 3). However, the addition of different concentration of NaCl with storage improves the stability of the flour emulsion because the emulsion droplet size decreased with the increased NaCl concentration (Figure 4.48), which is an indication of emulsion stability. Within droplet size measured for different NaCl concentration, the least droplet size was observed on day 1 at 4% w/v NaCl addition. Thus, apart from day 1, the least droplet size was observed at 10% w/v NaCl addition on storage day 2 to day 5, while NaCl concentration of 4% w/v resulted in highest droplet size on storage day 2 to day 5 ranged from 0.0355 ± 0.000 µm on day 2 and 0.0450 ± 0.0014 µm on day 5.

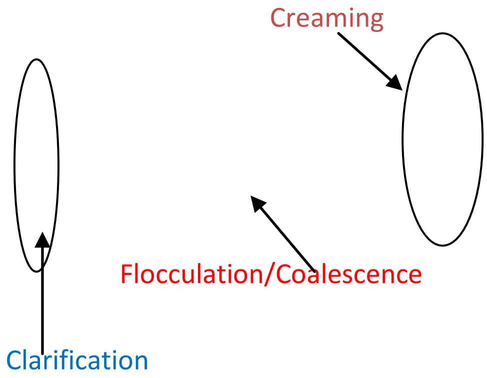


Figure 4.46 Effect of NaCl on BGNF Stabilized emulsion on day 1

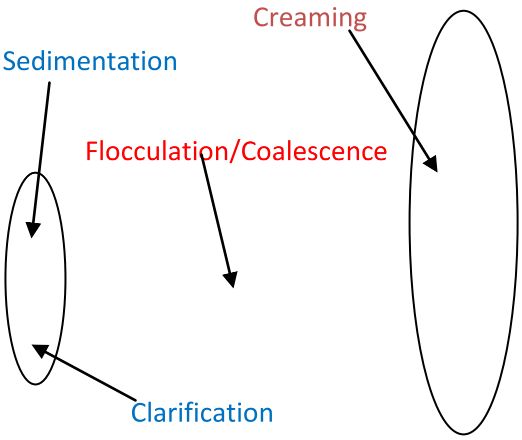


Figure 4.47 Effect of NaCl on BGNF Stabilized emulsion on day 5

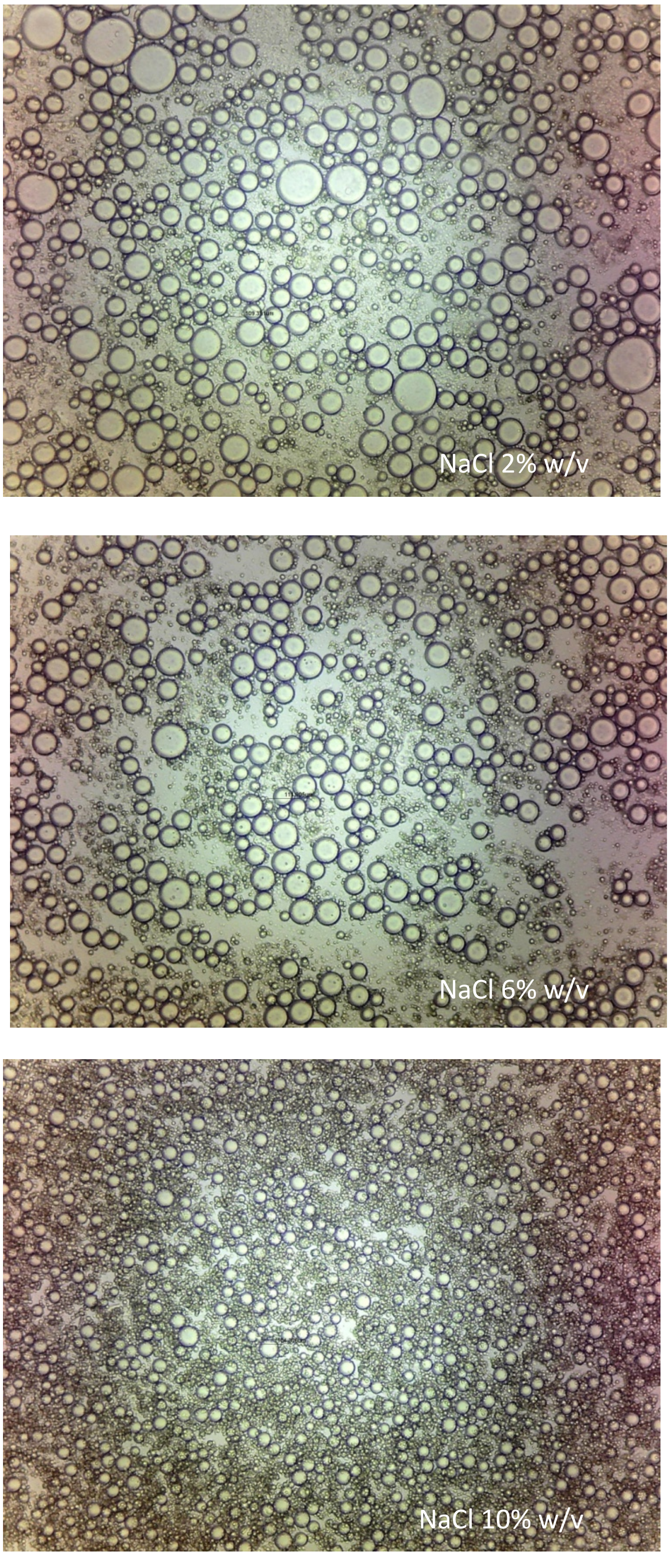


Figure 4.48 Effects of NaCl concentration on oil droplet size of BGNF stabilized emulsion

On comparing the overall mean droplet size value measured between storage duration period, day 1 had the least, 0.0075 ± 0.0062 µm while the mean droplet size on day 5 was the highest, 0.0331 ± 0.0826 µm. Thus emulsion stability of the flour was maximum on day 1 compared with day 5. The emulsion droplet size measured after each storage period was however significantly ($p < 0.05$) increased. This implies that the stability of flour emulsion in the NaCl medium were affected by storage and decreased with increase in storage duration. Also the mean droplet sizes of the emulsion were significantly different ($p < 0.05$) at the addition of different NaCl concentration between 2% w/v to 8% w/v, however the droplet sizes were not significantly different ($p > 0.05$) between 8% w/v and 10 % w/v NaCl addition.

Although, such effects may vary depending on the nature of emulsion and the emulsifier used, it is interesting to observe that the combination of BGNF and NaCl in food emulsions for example, may not negatively affect the stability. In fact, increased NaCl concentration enhances the emulsion stability. In a study conducted by Yang et al. (2006), to investigate the effect of salts on emulsion stability, the droplet sizes of the emulsions decreased with increased salt concentration. This observation was attributed to the favourable interaction between the electrolytes and the oil-water interface leading to enhanced adsorption of the emulsifier. This effect also decreased the creaming rate since creaming stability of emulsions is related to the emulsion droplet size. A further explanation offered as additional reason for increased stability of emulsions with increased salt concentration is the increased viscosity of the medium as a result of the increasing salt concentration (Yang et al., 2006). This was also observed in this study.

Aronson and Petko (1993) observed drastic changes in the properties of concentrated w/o emulsions when electrolyte was introduced into the aqueous phase at concentrations as low as 0.02 M. They reported that the rate of coarsening decreased, and coalescence of water droplets during the freeze/thaw process was inhibited. Long-term storage stability also improved with higher salt concentration. The authors proposed that the electrolyte-induced stability of the w/o emulsions was probably achieved by a higher adsorption density of the emulsifier by the fractionation of the concentrated electrolyte solution that wetted the ice crystals and prevented their fusion. The salt-containing emulsions also exhibited better rheological properties (Aronson and Petko, 1993).

4.6.3b Effects of NaCl on BGNS-stabilized emulsions

Storage time and NaCl had significant ($p < 0.05$) effect on the MR and droplet size of BGNS-stabilised emulsions. The storage time significantly ($p < 0.05$) increased the MR and droplet size. The interaction between storage time and NaCl had a significant (p <

0.05) effect on MR and the droplet size. This indicated that the influence of storage time differed within the salt range. Increase in NaCl decreased MR and droplet size (Table 4.24 and 25)

The control emulsion was the optima emulsions containing no NaCl. The influence of NaCl on the creaming stability of BGNS-stabilized emulsion is presented in Figure 4.49. The influence of the NaCl on the mean droplet diameter is presented in Figure 4.50. Similar to observations on the effects of NaCl on BGNF-stabilized emulsions, the presence of NaCl increased the stability of BGNS-stabilized emulsions in concentration-dependent manner.

Higher NaCl concentration resulted in higher degree of stability to creaming (Figure 4.49) and smaller mean droplet diameter (Figure 4.50). As observed earlier, the instability in BGNS-stabilized emulsions was generally higher than in the corresponding BGNF-stabilized emulsions. The effects of NaCl salt were maximum on day 1, at 2% w/v concentration with MR value 0.0145 ± 0.0002 mm/min. The MR observed at this concentration was the lowest and this indicated that the emulsion was most stable, while the MR (0.0216 ± 0.0038 mm/min) at 10% w/v was the highest. The least stable starch emulsion was observed in day 1, after which the MR of the other storage duration period showed a reverse pattern, with 4% w/v NaCl starch emulsion having the highest MR, while the 10% w/v NaCl starch emulsion had the least MR. There was a significant difference ($p > 0.05$) in the MR over the emulsion storage period. Although the mean MR at different NaCl concentration on day 1 was the least, MR observed over each of the storage period were significantly different ($p < 0.05$) from each other. This implies that increased NaCl concentration resulted in decreased MR, and this encouraged emulsion stability (Figure 4.51 and 4.52 show Turbiscan® destabilisation profiles for the effect of NaCl on emulsion stability, see more profiles are in Appendix 3). The mean droplet diameters measured immediately after homogenizations were similar in all the emulsion samples (Figure 4.50). This suggests that the presence of NaCl did not affect the initial emulsification process. The observed effect was therefore on the ability of the NaCl to prevent the destabilization of the BGNS-aided emulsification. The droplet size, 0.01050 ± 0.0007 µm at 4, 6, 8 and 10 g remained unchanged on day 1 except at 2% w/v NaCl which showed the least droplet size of 0.0100 ± 0.0000 µm though not significantly different from those of higher NaCl concentration. The overall mean of 0.0104 ± 0.0005 µm was observed in starch emulsion droplet size for 2% w/v to 10% w/v on day 1. However, the droplet size over day 2 to day 5 decreased with increased concentration of NaCl (Figure 4.53). The overall mean droplet size over the storage period, increased on the 5 days storage period i.e. 0.0104 ± 0.0005 µm for day 1 to 0.0440 ± 0.0187 µm for day 5.

Table 4.24 Tests of Between-Subjects effects for migration rate of BGNS emulsions prepared at varying NaCl concentration

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.090[b]	24	0.004	106.451	0.000
Intercept	0.302	1	0.302	8607.383	0.000
Day	0.070	4	0.017	497.704	0.000
NaCl	0.012	4	0.003	87.526	0.000
Day * NaCl	0.008	16	0.000	13.369	0.000
Error	0.001	25	3.507E-005		
Total	0.392	50			
Corrected Total	00.090	49			

[a] R Squared = .990 (Adjusted R Squared = .981)

Table 4.25 Tests of Between-Subjects effects for droplet sizes of BGNS emulsions prepared at varying NaCl concentration

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.013[b]	24	0.001	142.733	0.000
Intercept	0.034	1	0.034	8545.055	0.000
Day	0.007	4	0.002	446.167	0.000
NaCl	0.004	4	0.001	279.500	0.000
Day * NaCl	0.002	16	0.000	32.683	0.000
Error	9.828E-005	25	3.931E-006		
Total	0.047	50			
Corrected Total	0.014	49			

[a] R Squared = .993 (Adjusted R Squared = .986)

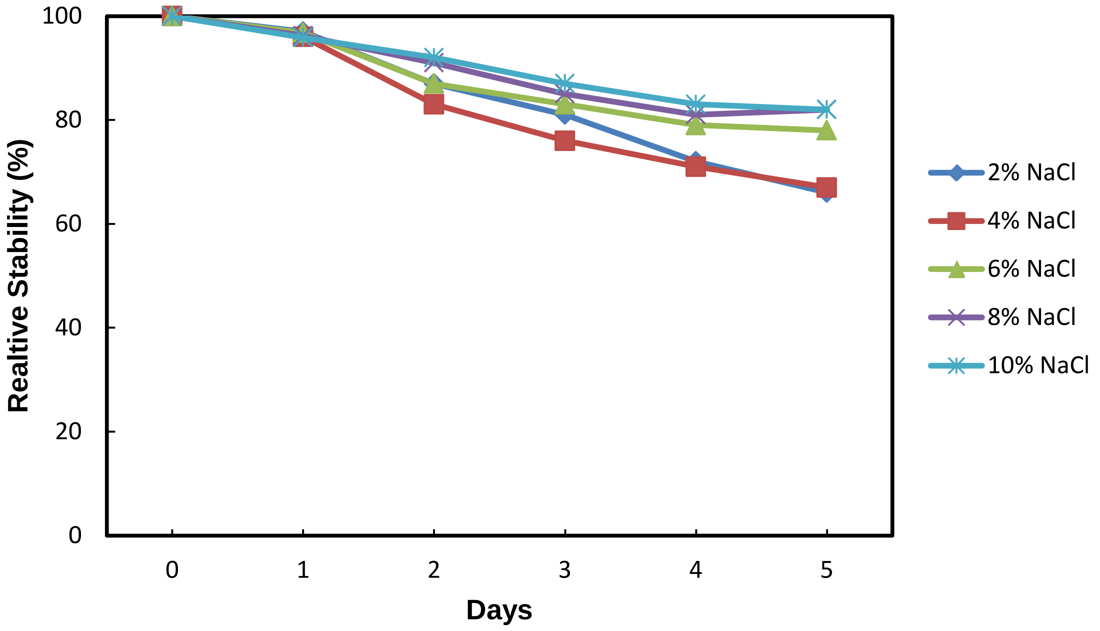


Figure 4.49 The influence of NaCl on the relative stability of BGNS-stabilized emulsions

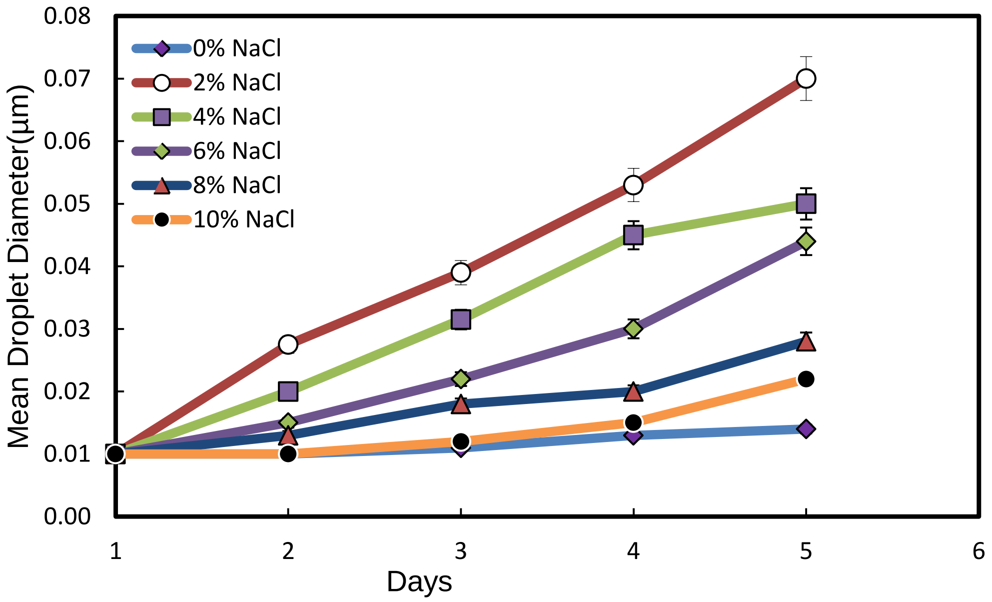


Figure 4.50 The influence of NaCl on the mean droplet diameter of BGNS stabilized emulsions

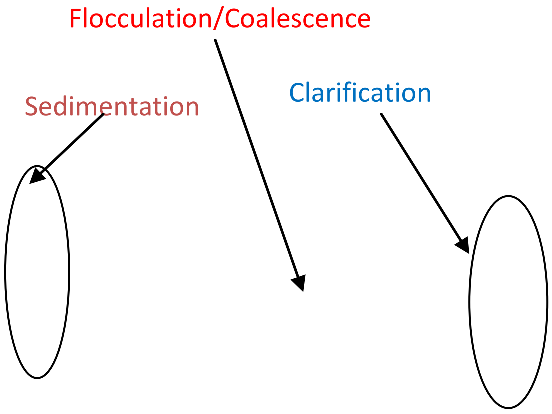


Figure 4.51 Effect of NaCl on BGNS stabilized emulsion on day 1

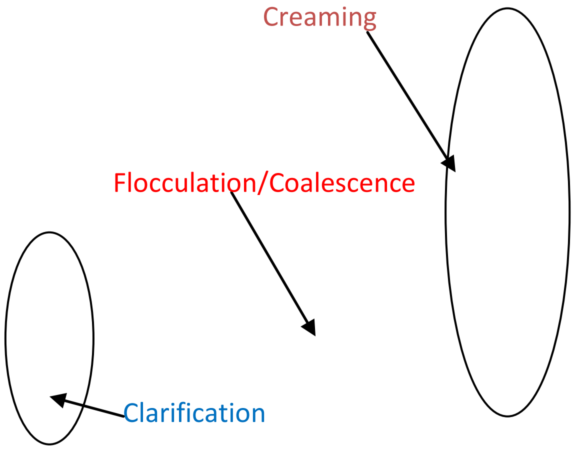


Figure 4.52 Effect of NaCl on BGNS Stabilized emulsion on day 5

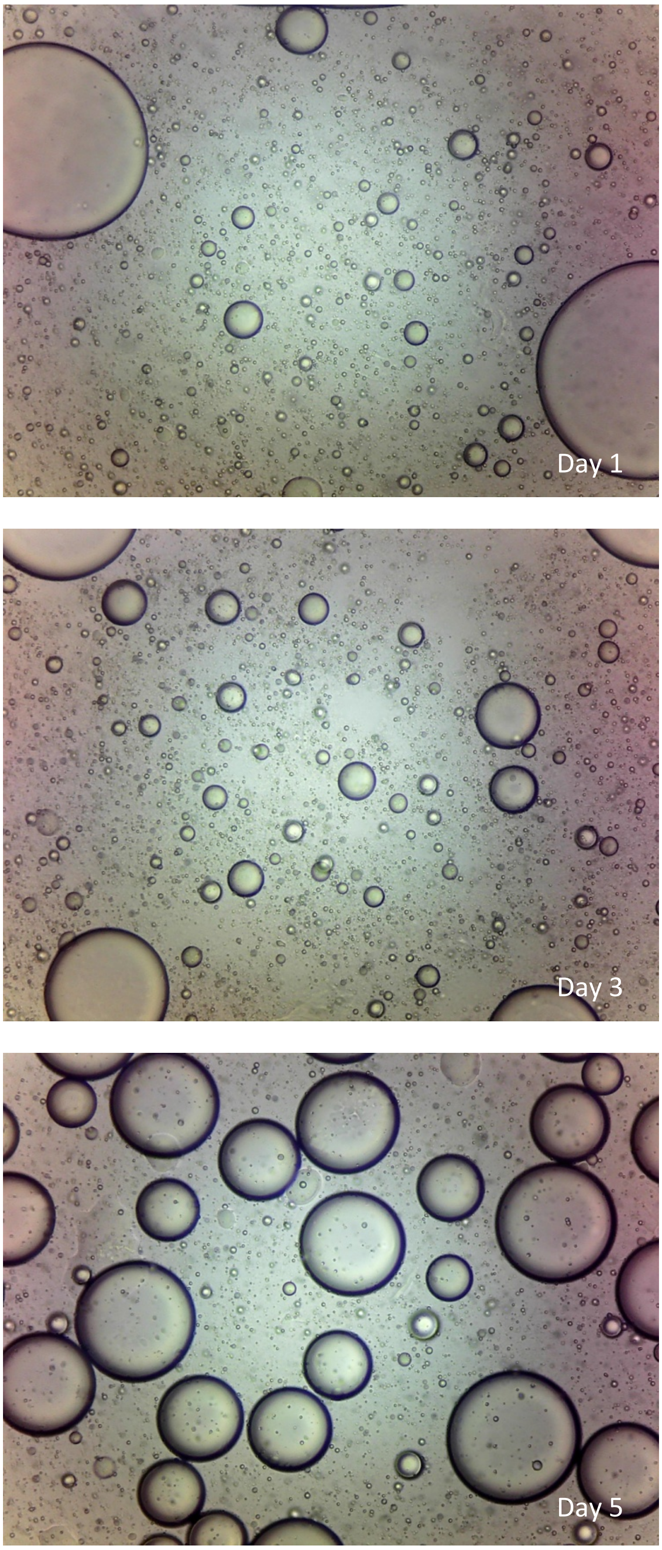


Figure 4.53 Effects of 10% NaCl concentration on oil droplet size of BGNS stabilized emulsion

In effect the stability of the starch emulsion decreased throughout the duration of storage. Thus, the droplet sizes observed for the different storage duration were significantly different ($p < 0.05$) from each other. The mean droplet size for the different NaCl concentrations were significantly different ($p < 0.05$) from one another, while the mean MR for the storage duration increased from day 1 to day 5, probably as a result of emulsion destabilization. It was interesting to observe that the combination of BGNS and NaCl in food emulsions for example, may not negatively affect the stability, and as can be seen, increased NaCl concentration enhanced the emulsion stability. As discussed earlier, (section 4.6.3b) this finding agrees with the literature.

4.6.4 Effects of vinegar on the emulsions

Table 4.26 and 4.27 show the influence of vinegar on the stability and droplet sizes of the BGNF- and BGNS-stabilized emulsions.

Table 4.26 Summary of the influence of stirring on the stability of BGNF- and BGNS-stabilized emulsion

	2%Vinegar		4%Vineagr		6%Vinegar		8%vinegar		10% Vinegar	
	Relative stability (%)									
DAYS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS
0	100	100	100	100	100	100	100	100	100	100
1	96.3	95	93.2	93	94	91	92.1	90	90	88
2	92	86	89	84	84	70	81	64	77	58
3	88	80	85	72	79	58	74	47	66	35
4	84	81	81	66	75	50	69	39	57	24
5	81	64	78	59	73	46	66	32	49	17

Table 4.27 Summary of the influence of stirring on the droplet size of BGNF- and BGNS-stabilized emulsion

	2%Vinegar		4%Vineagr		6%Vinegar		8%vinegar		10% Vinegar	
	Droplet size (µm)									
	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS	BGNF	BGNS
0	7.00E-03	0.01	7.00E-03	0.01	8.00E-03	0.01	8.00E-03	0.01	8.00E-03	0.01
1	7.00E-03	0.01	7.00E-03	0.01	8.00E-03	0.01	8.00E-03	0.01	8.00E-03	0.01
2	0.1	0.011	0.12	0.019	0.13	0.029	0.165	0.037	0.183	0.04
3	0.15	0.012	0.18	0.034	0.19	0.043	0.196	0.067	0.22	0.07
4	0.185	0.013	0.22	0.05	0.249	0.068	0.255	0.084	0.265	0.09
5	0.22	0.013	0.25	0.082	0.26	0.094	0.265	0.101	0.272	0.11

4.6.4a Effects of vinegar on BGNF-stabilized emulsions

Storage time and vinegar concentration had significant ($p < 0.05$) effect on the MR and droplet size of BGNF-stabilised emulsions. The storage time and the vinegar concentration significantly ($p < 0.05$) increased the MR and droplet size. The interaction between storage time and vinegar had a significant ($p < 0.05$) effect on MR and the droplet size. This indicated that the influence of storage time differed within the vinegar concentration range (Table 4.28and 4.29).

The relative creaming stability of BGNF-stabilized emulsions was drastically reduced in a concentration-dependent manner by vinegar. The study result showed that the MR increased with increase in the volume of vinegar for the storage period. However, the mean MR for each day showed that MR for day 1 was the least 0.0298 ± 0.0102 mm/min and this progressively increased until day 5 with MR of 0.1489 ± 0.0567 mm/min. Hence, increase in the concentration of vinegar added to the emulsion, thus resulted in the increased MR. Therefore, the addition of vinegar tends to lower the emulsion stability (Figure 4.54 and 4.55 show Turbiscan® destabilisation profiles for the effects of vinegar on the emulsions. More profiles are shown in Appendix 4)

Concentration of vinegar, as low as 2% v/v, reduced stability by 20% day 5 (Figure 4.56). Similarly, 10% v/v vinegar caused about 50% reduction in relative stability by day 5 (Figure 4.56). The presence of increasing concentration, generally, led to increased migration rate and the destabilization of the emulsion. The day 1 droplet migration rate also showed significant difference in the presence. As measured immediately after homogenization, the droplet migration rate was about 10% higher in the presence of 10% v/v vinegar than the control emulsion containing no vinegar. This may not necessarily suggest that the presence of vinegar inhibits emulsification process. This is because, as shown in Figure 4.56, the oil phase was broken into droplets of similar mean diameter after the homogenization. The mobility and attraction of the oil droplet towards one another might have been aided by the presence of vinegar (Yilmazer et al., 1991).

Table 4.28 Tests of Between-Subjects effects for migration rate of BGNF emulsions prepared at varying vinegar concentration

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.162[b]	24	0.007	100.184	0.000
Intercept	0.479	1	0.479	7121.338	0.000
Day	0.089	4	0.022	329.121	0.000
Vinegar	0.059	4	0.015	217.926	0.000
Day * Vinegar	0.015	16	0.001	13.515	0.000
Error	0.002	25	6.732E-005		
Total	0.643	50			
Corrected Total	0.164	49			

[b] R Squared = .990 (Adjusted R Squared = .980)

Table 4.29 Tests of Between-Subjects effects for droplet sizes of BGNF emulsions prepared at varying vinegar concentration

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.408[b]	24	0.017	237.074	0.000
Intercept	1.339	1	1.339	18663.975	0.000
Day	0.382	4	0.096	1331.952	0.000
Vinegar	0.019	4	0.005	65.482	0.000
Day * Vinegar	0.007	16	0.000	6.252	0.000
Error	0.002	25	7.175E-005		
Total	1.749	50			
Corrected Total	0.410	49			

[b] R Squared = .996 (Adjusted R Squared = .991)

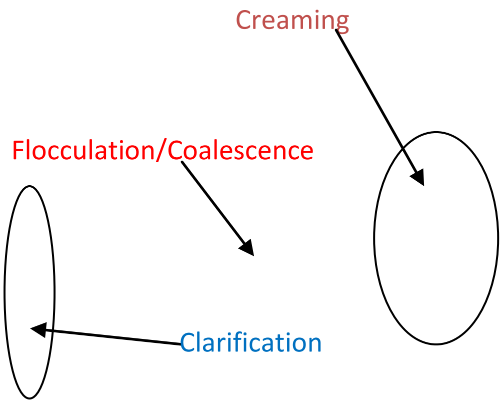


Figure 4.54 Effect of vinegar on BGNF Stabilized emulsion on day 1

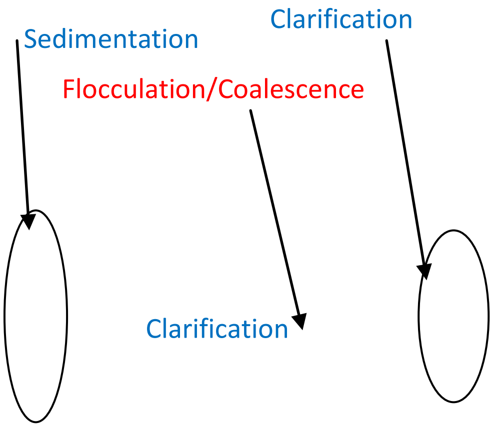


Figure 4.55 Effect of vinegar on BGNF Stabilized emulsion on day 5

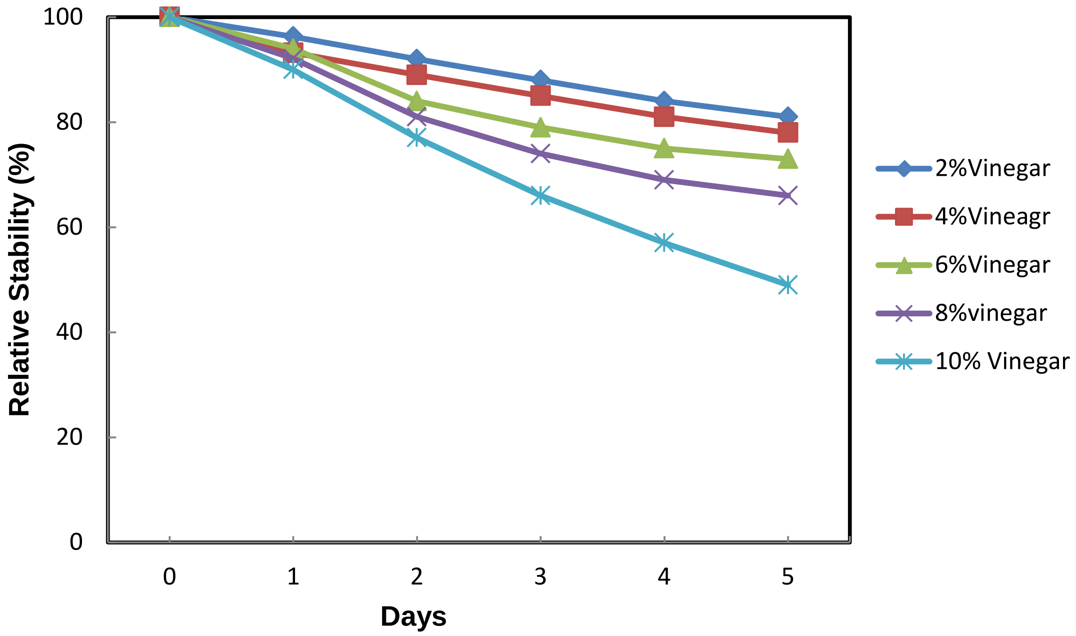


Figure 4.56 The influence of vinegar on the relative stability of BGNF-stabilized emulsions

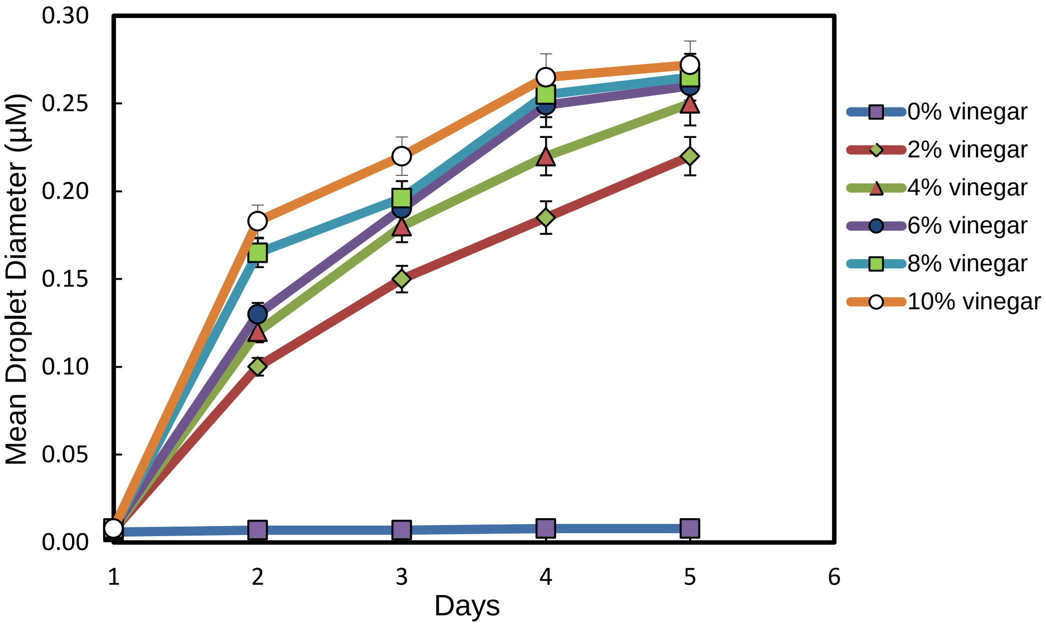


Figure 4.57 The influence of vinegar on the mean droplet diameter of BGNF-stabilized emulsions

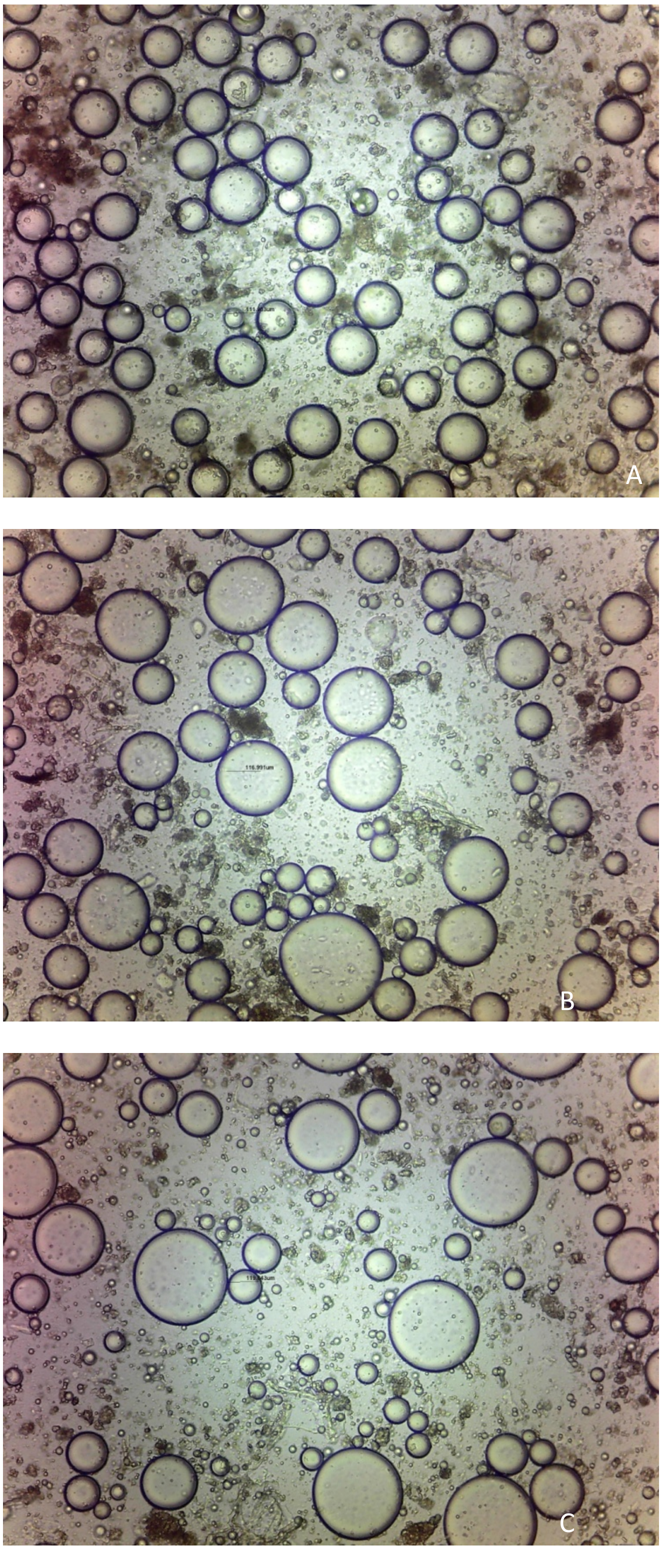


Figure 4.58 Effect of 6% vinegar concentration on oil droplet size of BGNF stabilized emulsion on (a) day 1, (b) day 3, and (c) day 5

The destabilizing effect of vinegar was also evident on the mean droplet diameter as observed over days 1 to 5 (Figure 4.57). The initial mean droplet diameters were not affected by the presence of vinegar, suggesting that vinegar did not affect the emulsification process, but stability. Increasing vinegar concentration also resulted in increase in droplet size for each emulsion after each storage period (Figure 4.58). The droplet size on day 1 showed that emulsion with vinegar concentration of 2% v/v had the least droplet size value (0.0690 ± 0.0004 µm), while 10% v/v vinegar had the highest droplet size (0.0810 ± 0.0014 µm). The droplet size observed on day 5 showed that emulsion with 2% v/v vinegar had the least droplet size, 0.2195 ± 0.0636 µm while the highest droplet size of 0.2705 ± 0.0120 µm was observed for 10% vinegar. In general, the overall mean droplet size in day 1 was the least (0.0765 ± 0.0005µm) and the highest (0.2528 ± 0.0202 µm) for day 5. Thus, the addition of vinegar to the flour emulsion resulted in increase in droplet size and thus reduced the emulsions stability. Significant differences ($p < 0.05$) existed in the droplet size of the emulsion with vinegar concentration, and in the droplet size over the storage period. Vinegar is acidic. Its presence in the emulsion will lower the pH. The findings thus, agree with the observed pH effects, BGNF-stabilized emulsions degraded faster with decreasing pH. The findings with pH and vinegar are thus consistent with each other

4.6.4b Effects of vinegar BGNS-stabilized emulsions

Storage time and vinegar concentration had significant ($p < 0.05$) effect on the MR and droplet size of BGNS-stabilised emulsions. The storage time and vinegar concentrations significantly ($p < 0.05$) increased the MR and droplet size. Interaction between storage time and vinegar concentration had a significant ($p < 0.05$) effect on MR and the droplet size. This indicates that the influence of storage time differ within the vinegar concentration range (Table 4.30 and 4.31).

Much more than its effect on BGNF-stabilized emulsions, the presence of vinegar accelerated the destabilization of BGNS-stabilized emulsions (Figures 4.59 and 4.60). There was over 80% phase separation of the BGNS-stabilized emulsions containing 10% vinegar, by day 5. The extent of instability caused by the presence of vinegar in the BGNS-stabilized emulsions was generally twice the observed instability in the corresponding emulsions stabilized by BGNF. Both migration rate and the droplet size growth showed that vinegar reduced the stability of the emulsions. The statistical analysis also showed that the addition of vinegar appeared to have a linear relationship with increase in the concentration of vinegar, the higher the vinegar added the higher the MR of the emulsion. At different vinegar concentration, the emulsion stability decreased with

Table 4.30 Tests of Between-Subjects effects for migration rate of BGNS emulsions prepared at varying vinegar concentration

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.532[b]	24	0.022	150.265	0.000
Intercept	1.502	1	1.502	10178.794	0.000
Day	0.321	4	0.080	543.465	0.000
Vinegar	0.175	4	0.044	296.248	0.000
Day * Vinegar	0.037	16	0.002	15.470	0.000
Error	0.004	25	0.000		
Total	2.038	50			
Corrected Total	0.536	49			

[b] R Squared = .993 (Adjusted R Squared = .987)

Table 4.31 Tests of Between-Subjects effects for droplet size of BGNS emulsions prepared at varying vinegar concentration

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.050[b]	24	0.002	183.207	0.000
Intercept	0.137	1	0.137	12061.200	0.000
Day	0.044	4	0.011	962.520	0.000
Vinegar	0.004	4	0.001	93.867	0.000
Day * Vinegar	0.002	16	0.000	10.713	0.000
Error	0.000	25	1.140E-005		
Total	0.188	50			
Corrected Total	0.050	49			

[b] R Squared = .994 (Adjusted R Squared = .989)

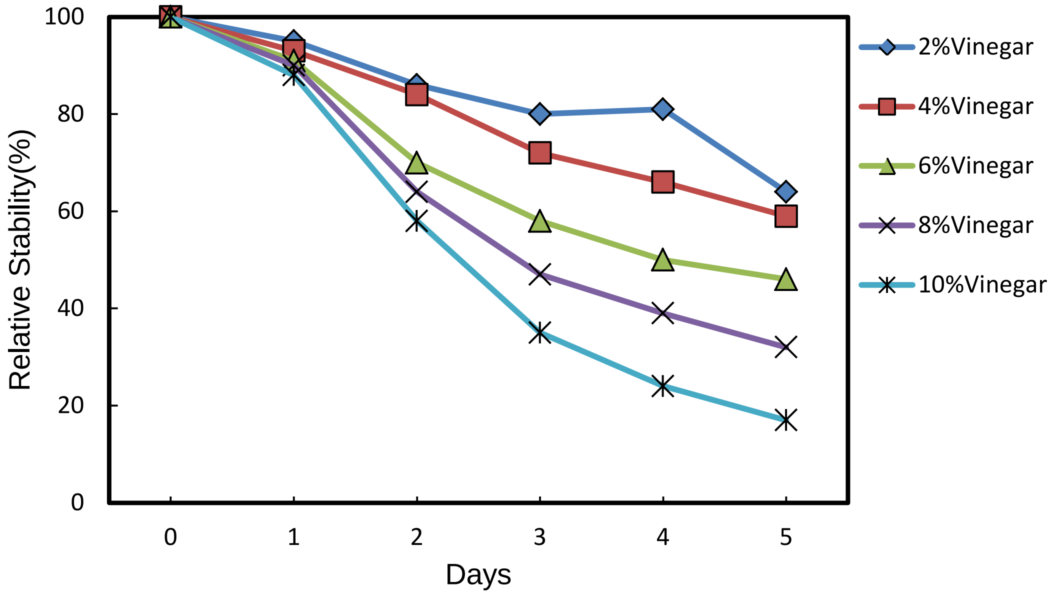


Figure 4.59 The influence of vinegar concentration on the relative stability of BGNS-stabilized emulsions

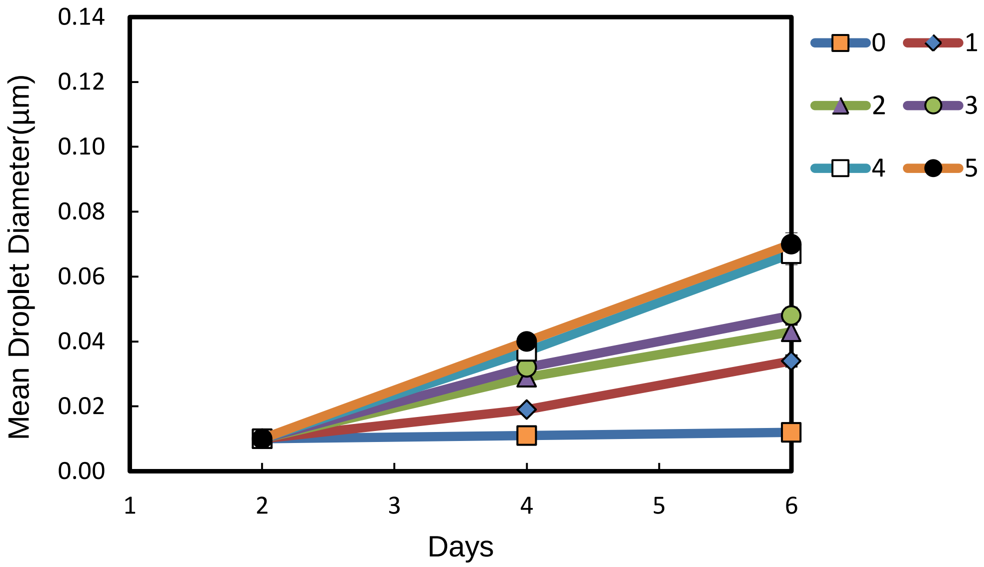


Figure 4.60 The influence of vinegar on the mean droplet diameter of BGNS-stabilized emulsions

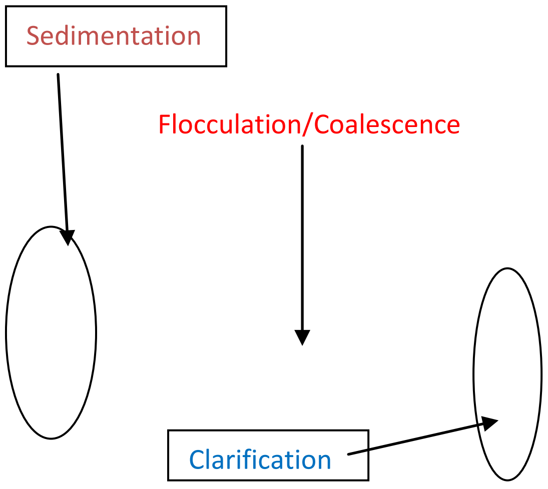


Figure 4.61 Effect of vinegar on BGNS Stabilized emulsion on day 1

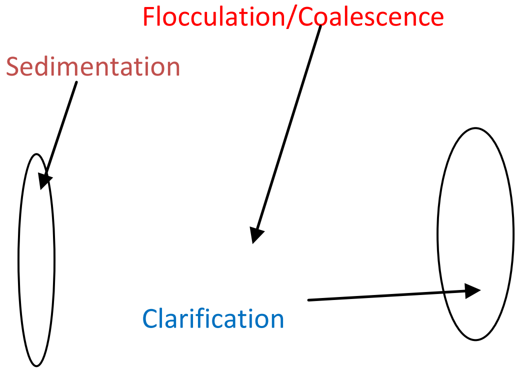


Figure 4.62 Effect of vinegar on BGNS Stabilized emulsion on day 5

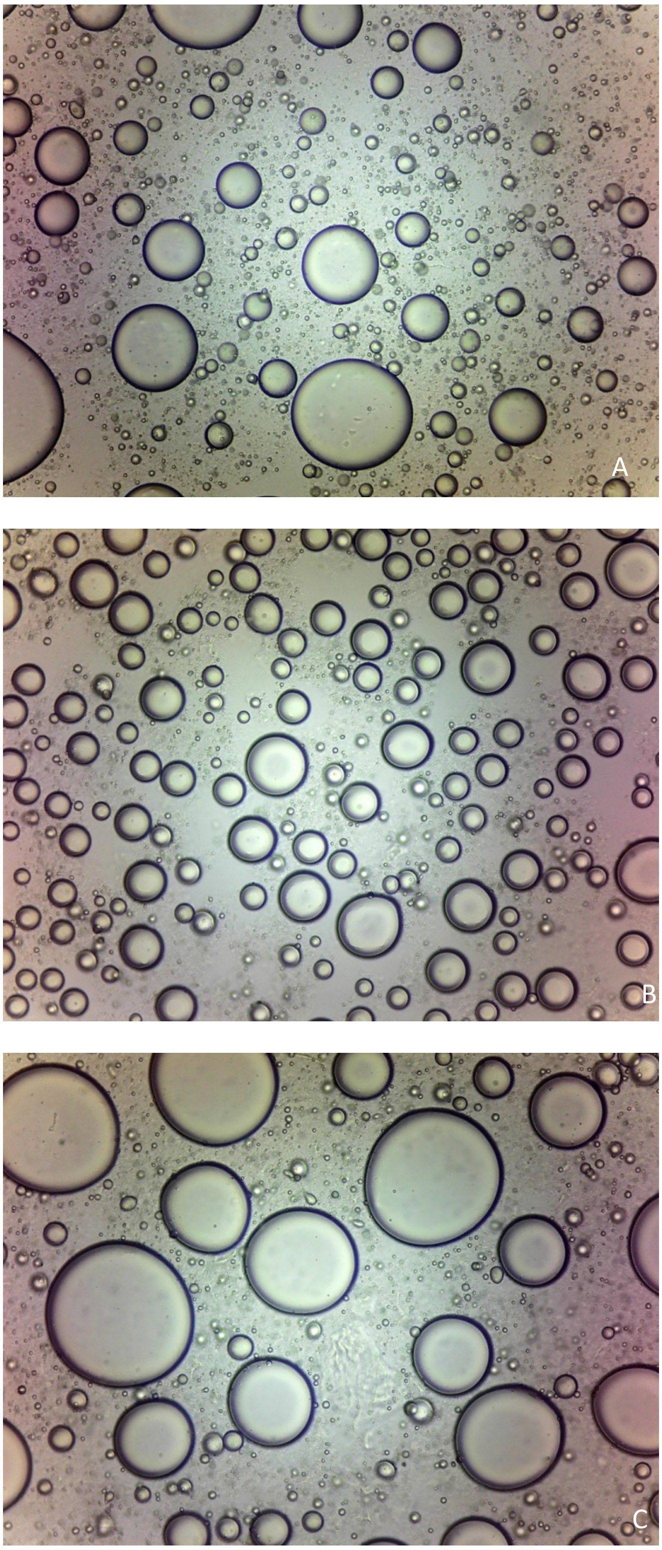


Figure 4.63 Effect of 6% vinegar concentration on oil droplet size of BGNS stabilized emulsion on (a) day 1, (b) day 3, and (c) day 5

storage period (Figure 4.61 and 4.62 show the Turbiscan® destabilisation profiles of the effect of vinegar on the emulsions, more profiles are available in appendix 4).

The mean MR value for starch emulsion at day 5 was the highest 0.2610 ± 0.0133 mm/min; hence the emulsion was most stable on day 1 with least MR of 0.0386 ± 0.0133 mm/min. The mean MR of the emulsion obtained from daily evaluations, over day 1 to day 5, were significantly different ($p < 0.05$) from one another. The mean MR obtained for the different concentration of vinegar within daily subset were also significantly different ($p < 0.05$) from one another. The addition of different concentration of vinegar did not show any effect on the droplet size of the starch emulsion in day 1. However, the droplet size within the concentration cluster for storage duration of day 2 to day 5 increased with increased vinegar concentration for each storage period of the starch emulsion (Figure 4.63). The overall mean droplet size between each of the cluster of storage period showed a general increase in droplet size with day 1 showing the least droplet value of 0.0150 ± 0.0070 µm and day 5 having the highest droplet value of 0.0944 ± 0.0086 µm. There were however, significant differences ($p<0.05$) in the droplet size values between the storage. Also the effect of vinegar concentration on droplet size of the starch emulsion was significantly different for the 2%v/v to10%v/v added, except at vinegar concentration of 4% v/v and 6% v/v which showed some degree of consistency.

Legumes are rich sources of energy, dietary fiber, protein, minerals and vitamins (Jacobs and Gallahe, 2004). This makes them important diet in resource-poor countries where animal protein is unavailable or unaffordable, and in certain cases, forbidden due to religious or cultural factors. In addition, legumes, like other plant produce are health-friendly especially in cardiovascular diseases (Flight and Clifton, 2006). The current study on BGN, a leguminous produce consumed in Africa, explores its potential for use in food products as emulsifying agent. With a good blend of starch and protein, BGN has shown emulsifying potential. Its use for emulsification purpose will provide additional nutrient in the emulsion. For example, protein isolate from BGN has been shown to be rich in essential amino acids especially lysine (7.33%) and leucine (9.05%) and non-essential amino acids - glutamic acid (14.74-15.56%) and aspartic acid (12.37- 12.81%) (Mune et al., 2011). This rich nutritional constituents and the emulsifying property shown in the current study can be harnessed to increase the utility of BGN in food products and supplements. This study has therefore, been able to validate the hypothesis that BGNF and BGNS are capable to stabilize emulsions, and make such emulsions withstand some pH changes, salinity differences and storage.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In line with the set objectives, this study has been able to assess the ability of BGNF and BGNS to stabilize oil-in-water emulsions and the following were the conclusions:

1. Emulsion containing 9% w/v BGNF with 39% w/v oil for BGNF and 5% w/v BGNS with 30% w/v oil for BGNS gave the optimal emulsion.
2. The optimal emulsions were stable till day 5 of storage duration.
3. Homogenization speed of 15000 rpm gave a satisfactory emulsion.
4. Emulsion stability increased with increasing concentration of salt (NaCl).
5. Increasing concentration of vinegar in the emulsions decreased the stability.
6. Emulsion stability improves with increasing pH till pH 7, beyond which stability decreases.

In general, BGNF appeared to be a better emulsifier/stabilizer than the BGNS. This may be due to the multiple constituents of starch, protein and ash in BGNF compared to BGNS which contains starch only. The hypothesis tested in this study is valid as both BGNF and BGNS demonstrated emulsifying/stabilizing properties.

5.2 Recommendation for further study

In view of the current findings, it may be interesting and scientifically justified to explore further studies with BGN. Such studies may include:

1. Chemical analysis of the composition of different varieties of BGN. This will provide an answer to the questions of expected variation in the emulsifying properties of BGN of different varieties.
2. Investigation of the emulsifying properties of the other individual constituents of BGN – protein, ash, total carbohydrate etc.
3. In line with the increasing viscosity with time, observed in BGNF- and BGNS-stabilized emulsions, it will be interesting to investigate the ability of BGN and its products to serve as thickening agents in food products.
4. With the addition of preservatives, emulsions stabilized with BGNF and BGNS may last longer than 5 days, as microbial growth will be suppressed. The effect of such additive may be investigated for long-term stability of such emulsions.

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APPENDICES

Appendix 1

Turbiscan® profiles showing effect of stirring on the emulsion stability

Delta Back Scattering

Appendix 2

Turbiscan® profiles showing effect of pH on the emulsion stability

Appendix 3

Turbiscan® profiles showing effect of NaCl on the emulsion stability

Appendix 4

Turbiscan® profiles showing effect of vinegar on the emulsion stability