GUM ACACIA STRUCTURAL FEATURES AND EMULSION STABILITY

by

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(Under the Direction of Louise Wicker)

ABSTRACT

The structure-function relationship of commercial gum acacia (GA) from *Acacia senegal* (SG1, SG2 and SG3) and *Acacia seyal* (SY1 and SY2) were studied. The protein content was less than 0.9 μg/mg ($p>0.05$) and ζ-potential was less than -32 mV ($p>0.05$). The effective diameter was between 172 to 257 nm and in the order SG2<SG3<SG1 and SY2<SY1 for the two types gums. A linear regression relationship between sample concentration and 1-anilinonaphthalene-8-sulfonic acid (ANS) relative fluorescence intensity was observed, indicating surface hydrophobicity was significantly different in the order of SG2>SG3>SG1>SY1>SY2. Raman, FT-IR spectra peaks and peak ratios of protein to carbohydrate provided additional structural information. The stability of emulsions, prepared with the gums, during a 6-day storage study at 4, 25, 30ºC, indicated that emulsion stability was greater in SG than SY samples at the first day of storage. The rate of sedimentation, creaming and particle size changes varied between gums stabilized emulsions according to gum type, surface hydrophobicity, and effective diameter. Particle size distribution (PSD) increased as surface area or volume distribution for emulsions stabilized by SG or SY, respectively.

INDEX WORDS: Surface hydrophobicity, ζ-potential, Raman, FT-IR, PSD, Particle migration, Coalescence, Creaming

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DEDICATION

I would like to dedicate this thesis to my boy friend, Zheng Zhao, for all of his love and support; to my mom for her great encouragement and shoulder to cry on; and to my brother and sister for the understanding and encouragement.

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CHAPTER 1

INTRODUCTION

Gum acacia has been used as a food emulsifier or stabilizer for more than a decade, but its two botanical species, *Acacia senegal* and *Acacia seyal*, the structure, properties, and function performance are not fully understood. In the food industry, *Acacia senegal* is often regarded as "gold standard" natural hydrocolloid with better functionality as an emulsifier/stabilizer than *Acacia seyal* and is extensively used in beverage emulsions. In this study, five commercial gum products from both Acacia senegal samples (denoted as SG1, SG2 and SG3) and Acacia seyal samples (denoted as SY1 and SY2) are first characterized by physicochemical methods, fluorescence probes and spectroscopic techniques for their powder or solutions features, and then model emulsions are prepared with these five commercial gum products as the emulsifier/stabilizer and cold-pressured orange oil as the oil phase, and a 6-day storage study is performed under 4, 25 and 40 °C for the emulsion stability.

Gum is the exudation from acacia trees and Sudan is the world's largest producer with various grades gum, including hand picked selected, cleaned and sifted, cleaned, siftings, dust and red. Following export to Europe and the US, some grades of gum are processed providing greater quality and convenience to the users (Islam et al. 1997). This may be by mechanical grinding (kibbling) which breaks up the gum nodules into various specific sizes for faster dissolving. Further processing may involve dissolution and filtration of the gum to remove impurities, followed by spray drying or roller drying (Phillips and Williams 2000; Coppen 1995).

The mechanism of gum acacia as the emulsifier/stabilizer is that protein functions as hydrophobic portion linked covalently to polysaccharides, binds strongly to the oil-water interface to reduce the interfacial tension (Dickinson 2009). In the previous papers, sugars and proteins are often investigated to characterize the structures of gum acacia. It is reported that both species of gums are composed of the same sugars, including D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid and 4-O-methyl glucuronic acid. They are joined by the same chemical bonds in a branched structure consisting of β1-3 linked D-galactose core and β1-6 D-galactose branch points. Protein content in acacia senegal is 1.8-2.1 μg/mg and acacia seyal is 0.7-1.3 μg/mg (Al-Assaf et al. 2005; Hassan et al. 2005), which is mainly in arabinogalactan-protein fraction (AGP), with the accepted "wattle blossom" or glycoprotein structure model (Goodrum et al. 2000; Qi et al. 1991).

In this work, surface hydrophobicity using a Flouorolog-2 Spectrofluorometer combined with Raman and FT-IR spectroscopic techniques for functional groups are used to characterize gum products properties. The basic principle is 1,8 ANS (1-anilinonaphthalene-8-sulfonic acid) as a fluorescent probe, can track both internal and external hydrophobic side chains of proteins by binding them, which is reported as ANS fluorescence intensity (Gasymov and Glasgow, 2007). Emulsion destabilization process is evaluated and analyzed by using TURBISCAN and Malvern, combined with microscopic methods and visual observation in a 6-day storage study. The TURBISCAN is an easy way of detecting clarification, creaming or sedimentation in a sample without the need to dilute or disturb the sample. The Malvern measures particle size by diffraction light scattering, which is based on volume, not number of particles.

The objective of this study is to investigate commercial gum acacia structural properties and functional performance, providing useful information for the food industries.

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CHAPTER 2

LITERATURE REVIEW

Gum Acacia Definition

Gum acacia (or gum arabic) is defined by the FAO/WHO Joint Expert Committee for Food Additives (JECFA) as: "a dried exudate obtained from the stems and branches of *Acacia senegal* (L.) Willdenow or *Acacia seyal* (Farm. Leguminosae) " (FAO, 1999). It is a natural hydrocolloid and is extensively used as an emulsifier/stabilizer in beverage emulsions (Tan, 2004; McClements, 2005). There are two botanical species of acacia trees approved for commercial use as sources of gum acacia: *Acacia senegal* (known as gum hashab) and *Acacia seyal* (known as gum talha), and both grow across the Sahelian belt of Africa, principally Sudan (Mahendran, Al-Assaf, Baldwin, Williams, & Phillips, 2008; Chikami, Casadei, Coppen, Nour, & Cesareo, 1997). However, gum acacia derived from these species differ in emulsifying stability, with that from *Acacia senegal* generally being considered as the industry "gold standard" (Reiner, Reineccius, & Peppard, 2010).

Commercial Gum Acacia Collection and Processing

Exudate gum is collected as partially dried tears from the gum trees usually in November or December in Sudan (Verbeken, Dierckx, & Dewettinck, 2003). The collection is repeated at about 15-day intervals for up to five or six collections in total, depending on the weather conditions and the health of the gum tree itself (Imeson, 1992). After collection, the gum is cleaned and graded. In Sudan, the gum from *Acacia senegal* is devided into various grades, from the best quality to the worst as: Hand-picked selected, Cleaned and sifted, Cleaned, Siftings,

Dust and Red (Islam, Phillips, Sljivo, Snowden, & Williams, 1997). Since 1995, the gum from *Acacia seyal* has been categorized into three grades, Super, Standard clean, and siftings (FAO, 1995). After collection, the gum can be further processed into powdered forms before marketing, which is usually produced by breaking up large lumps into smaller granuels, dissolving the gum in water, removing impurities by filtration or centrifugation and spray-drying (Islam, Phillips, Sljivo, Snowden, & Williams, 1997; Verbeken, Dierckx, & Dewettinck, 2003). Therefore, the original grades of the gum and the processing methods might result in variability in commercial gum products.

Chemical Composition

Gum acacia consists of mainly three distinct fractions with different chemical structures and varying protein contents (Siddig, Osman, Al-Assaf, Phillips, & Williams, 2005; Randall, Phillips, & Williams, 1988; Williams, Phillips, & Randall, 1990).

The major component is a highly branched polysaccharide consisting of β-(1-3) galactose backbone with linked branches of arabinose and rhamnose that terminate in glucuronic acid, which is found in nature as magnesium, potassium and calcium salt (Dror, Cohen, & Yerushalmi-Rozen, 2006). It is referred to as the arabinogalactan fraction (AG), which represents about 89% of the gum and contains <1% protein. It has a molecular mass of $2.5\sim3\times10^5$ Da (Mahendran, Al-Assaf, Baldwin, Williams, & Phillips, 2008).

A second component is a higher molecular weight arabinogalactan-protein complex in which arabinogalactan chains are covalently linked to a protein chain through serine and hydroxyproline groups (Dror, Cohen, & Yerushalmi-Rozen, 2006). It is referred to as the arabinogalactan-protein fraction (AGP), which represents about 10% of the total and contains ~10% protein. The attached arabinogalactan in the complex contains about 13% glucuronic

acids. It has a molecular mass of $1\sim2\times10^6$ Da (Mahendran, Al-Assaf, Baldwin, Williams, & Phillips, 2008).

The third component is a glycoprotein that differs in its amino acid composition from that of the AGP complex. It represents only ~1% of the total but contains up to 50% protein, which is the smallest fraction having the highest protein content (Dror, Cohen, & Yerushalmi-Rozen, 2006). It is the glycoprotein (GP) with a molecular mass of about 2×10^5 Da (Mahendran, Al-Assaf, Baldwin, Williams, & Phillips, 2008).

The chemical characteristics may vary significantly depending on the geographical origin and age of the gum trees, climatic conditions, soil environment, and even on the place of exudation on the tree (Idris, Williams, & Phillips, 1998; Islam, Phillips, Sljivo, Snowden, & Williams, 1997). The chemical compositions of *Acacia senegal* gum, as obtained by Idris, Williams, & Phillips (1998), include 0.22-0.39% nitrogen, 1.5-1.6% protein, 39-42% galactose, 24-27% arabinose, 12-16% rhamnose, 15-16% glucuronic acid, and equivalent mass of 1.1-1.2 KDa.

Current Molecular Structure Models

The AGP fraction, although being a minor component, plays an important role in the structure and functionality of gum acacia. The AGP fraction structure has not been fully resolved, but currently there are two popular models used to describe it.

One is the "wattle blossom" model where five or six large carbohydrate blocks are described as being attached to a polypeptide chain composed of repeating sequences of 19 amino acids forming a compact spheroidal structure (Fincher, Stone, & Clarke, 1983; Goodrum, Patel, Leykam, & Kieliszewski, 2000). This model is supported by research that shows the molecular mass of this fraction decreased after the treatment with proteolytic enzyme, from 10^6 Da to 10^5

Da (Connolly, Fenyo, & Vandevelde, 1987). Over the years, numerous studies were conducted, suggesting that AGP molecules have a globular structure and thus supporting this wattle blossom model (Connolly, Fenyo, & Vandevelde, 1988; Idris, Williams, & Phillips, 1998).

An alternative is the glycoprotein model, proposed by Qi, Cynthia, & Derek (1991), which referred to that the polysaccharide-protein complex as a twisted hairy rope of 150 nm length and 5 nm diameter, in which polysaccharide chains attached in regular intervals are twined around the protein backbone of some 400 amino acid residues. This model was supported by the electron micrographs of rotary shadowed GAGP molecules and chromatographic techniques done by their group.

Solution Properties

Gum acacia has a high solubility with fast hydration, low viscosity, and not sensitive to pH. It can readily dissolve in cold or hot water in high concentrations up to 50% with the common usage level around 10-20% (Verbeken, Dierckx, & Dewettinck, 2003). Due to its compact branched structure and therefore with small hydrodynamic volume, gum acacia solutions are characterized by a low viscosity, allowing the use of high gum concentrations in various applications (Dziezak, 1991). In addition, gum acacia has excellent emulsifying properties, particularly credited to the AGP fraction, which only accounts for a small fraction. Therefore, the total concentration required to produce a stable emulsions is much higher than for other protein emulsifiers (Williams & Phillips, 2000; Williams, Phillips, Stephen, & Churms, 2006). Solutions exhibit Newtonian behavior at concentrations up to 40% and become pseudoplastic at higher concentrations. The pH of the solutions is normally around 4.5-5.5, but maximal viscosity is found at pH 6.0 (Verbeken, Dierckx, & Dewettinck, 2003). Usually gum acacia works well at a broad range of acidic pH values.

Food Applications

The use of gum acacia in foods is mainly in the confectionery industry, where it is incorporated in a wide range of products to behave as the formulation aid, stabilizer/thickener or surface-finishing agent (Verbeken, Dierckx, & Dewettinck, 2003). It has a long tradition of use in wine gums, where it produces a clarity that is higher than other hydrocolloids (Williams & Phillips, 2000), and it prevents sucrose crystallization, provides a controlled flavor release, and slows down melting in the mouth, which makes the wine gum long-lasting in flavor (Imeson, 1992). In addition, it provides the appropriate texture to these candies, which are much easily deformed in the mouth but do not adhere to the teeth when chewed. In lower calories candies, gum is used to compensate for the loss of texture and mouthfeel, resulting from the replacement of sugars by artificial sweeteners. In chewing gums, it is used as a coating agent and as a pigment stabilizer (Huzinec & Graff, 1987). It is used in toffees and caramels as an emulsifier, to maintain a uniform distribution of the fat across the product. Gum acacia glazes are used for coatings for nuts, dragees, and others.

It is widely used as an emulsifier in the manufacture of soft drinks. Due to its stability in acidic conditions and high solubility, gum acacia is well suited for use in citrus and cola flavor oil emulsions. It can also form a stable cloud in the drink, imitating the effect of added fruit pulps and juices (Eng & Mackenzie, 1984), can also be used as a foaming agent and to assist lacing in beer (Tiss, Carriere, & Verger, 2001). In low-calorie and dietetic beverages, gum acacia can be used as a source of soluble fiber (Phillips, 1998). In powdered beverage mixes, it is added to produce the same opacity, appearance, mouthfeel, and palatability as the natural fruit juices (Verbeken, Dierckx, & Dewettinck, 2003). In microencapsulation, it can be used as the protective layer to be coated with liquid, solid or gaseous substances to prevent chemical

deterioration and the loss of volatile compounds (Verbeken, Dierckx, & Dewettinck, 2003). Microencapsulation is a useful technique to convert liquid food flavors to flowable powders that can be used in dry food products (McNamee, O'Sullivan, & O'Riordan, 1998). Due to high water solubility, low viscosity, and emulsification properties, gum acacia is an effective encapsulation agent and can be used in soups and dessert mixes. It can inhibit the extraction of proteins from the meat into the gravy, so it is also used to prevent gelation in canned gravy-based pet foods (Verbeken, Dierckx, & Dewettinck, 2003).

Oil-in-Water Emulsion Definition

An emulsion is composed of two immiscible liquids (usually oil and water), with one of the liquids being dispersed as small spherical droplets in the other liquid (McClements, 2005). In foods, the diameter of the droplets usually lie between 0.1 um to 100 um (Friberg & Larsson, 1997; Dickinson, 1992). Emulsions can be classified based on the relative spatial distribution of the oil and water phases. Oil-in-Water (O/W) emulsion refers to oil droplets dispersed in an aqueous phase, such as milk, cream, dressings, beverage, dips and sauces. Water-in-Oil (W/O) emulsion refers to water droplets dispersed in an oil phase, such as margarine and butter. The material that makes up the droplets is called dispersed, discontinuous, or internal phase, whereas the material that makes up the surrounding liquid is called dispersing, continuous or external phase. It is also possible to create various types of multiple emulsions, such as oil-in-water-in-oil (O/W/O), water-in-oil-in-water (W/O/W), oil-in-water-in-water (O/W/W) (Kim, Decker, & McClements, 2006).

Basic Preparation Method

The process of converting bulk oil and bulk water into an emulsion, or of reducing the size of the droplets in a preexisting emulsion, is known as homogenization. It can be carried out

by using the homogenizer, such as a high shear mixer, a high pressure valve homogenizer, a colloid mill or an ultrasonic homogenizer, to apply intense mechanical agitation to a liquid mixture (McClements, 2005).

Conventional emulsions are inherently thermodynamically unstable systems because the contact between oil droplets and water molecules is unfavorable, and they are intended to breakdown over time (Dickinson, 1992; Friberg, Larsson, & Sjoblom, 2004). In this case, in order to prepare emulsions that are kinetically stable over time (a few days, weeks, months or years), emulsifiers and stabilizers are required. An emulsifier is a surface-active substance that adsorbs to the surface of emulsion droplets to form a protective coating that prevents the droplets from aggregating with one another, but they do have different emulsifying mechanisms. Gum acacia, an amphiphile that is a mixture of anionic polysaccharides and protein fraction, tends to stabilize emulsions primarily through steric interactions (Garti & Leser, 2001; Castellani, et al., 2010; Wilde, 2000; Dickinson, 2003). Octenyl succinic anhydride (OSA) modified starch, is composed of starch molecules that have been chemically reacted with octenyl succinic anhydride to give some hydrophobic character (Tan, 2004; Given, 2009). Whey protein isolate is a mixture of amphoteric globular protein and polysaccharides, and tends to stabilize emulsions primarily through electrostatic interactions (Wilde, 2000; Chanamai & McClements, 2002).

Emulsion Preparation Parameters and Conditions

For freshly prepared concentrated emulsion, formulation composition and homogenization conditions are two main aspects determining emulsion quality (McClements, 2005). Formulations are composed of oil, preservative, deionized water, gum emulsifier, and/or weighting agent, especially the ratio of oil to emulsifier is critical in the formulation. Homogenization conditions include homogenization time, pressure and numbers of passes. For

further diluted beverage emulsion, besides formulation composition and homogenization conditions, pH is also a key factor, and citric acid is often used to adjust final pH (McClements, 2005).

Several researchers have studied emulsion preparation with gum acacia in previously published papers. In this study, we will focus on the freshly prepared concentrated emulsion, so a brief summary of concentrated emulsion preparation parameters and conditions is listed in Table 1.1 as follows.

Table 2.1 A brief summary of concentrated emulsion preparation parameters and conditions

Formulation composition (on a weight base)	Homogenization condition	Final droplet size (μm)	Authors
Cold-pressed orange oil 6.5%; Gum acacia 20.0%; Sodium benzoate 0.1%; Deionized water 73.4%	Gum solution hydrated for 24 hrs, pre-mixed at 50% full powder for 5 min using a bench-top high speed mixer, adjusted pH to 3.5, then fine homogenized at 13,000 psi in a single pass using a high pressure Microfluidizer	< 1 μm	Buffo, R.A. & Reineccius, G.A. 2002
Orange oil 5.0%; Gum acacia 10.0%; sodium azide 0.01%; Deionized water 84.99%	Pre-mixed for 2 min using a high speed blender, high-pressure homogenized at 9,000 psi in three passes using high pressure Microfluidizer	<1 μm	Qian, C., Decker, E.A., Xiao, H., & McClements, D.J. 2011
Cold-pressed orange oil 6, 8, 10%; Gum acacia 7, 10, 13%; Xanthan gum 0.1, 0.2, 0.3%; sodium benzoate 0.1%, Potassium sorbate 0.1%, citric acid 0.4%; Deionized water was added to make a 100% solution	Pre-mixed sodium benzoate, potassium sorbate and citric acid for 3 min using a high shear blender at 60°C, 24 hrs hydration, then added xanthan gum and orange oil and pre-mixed for another 1 min, high pressure homogenized at 4350, 4060 and 3625 psi for three passes	<1 μm	Mirhosseini, H., Tan, C.P., Hamid, N.S.A., & Yusof, S. 2008; Mirhosseini, H., Tan, C.P., Hamid, N.S.A., Yusof, S. & Chern, B.H. 2009; Mirhosseini, H., Tan, C.P., Hamid, N.S.A. and Yusof, S. 2008
Coconut oil 20%; SUPER GUM 6, 7, 8, 10, 12% for rotor-stator machine, and 8% for high pressure machine; Deionized water was added to make a 100% solution	One method was coarse mixed for 2 min at 17,500 rpm using a rotor-stator machine. The other method was after course mix, high-pressure homogenized at 2900 psi for two passes	<10 μm	Tipvarakarnknno, T., Einhorn-Stoll, U. & Senge, B. 2010

Canola oil 10%; Gum acacia 10%; sodium azide 0.01%; Deionized water 79.99%	Gum solution hydrated for 24 hrs, coarse mixed for 20 min at 9500 rpm using a rotor-stator machine, high pressure homogenized at 18,000 psi for one single pass with a Y-type (75 um) and another 200 um chambers to break down and relax droplets within 1 um	<1 μm	Klein, M., Aserin, A., Svitov, I. & Garti, N. 2010
Rice bran oil 5%; Gum acacia 10%; Sodium azide 0.001%; Deionized water 84.999%	Gum solution was stirred for 24 hrs, pre-mixed for 2 min using a high speed blender, high pressure homogenized at 9320 psi for three passes with a Y-type (75 um) chamber to break down droplet size	<10 μm	Charoen, R., Jangchud, A., Jangchud, K., Harnsilawat, T., Naivikul, O. & McClements, D.J. 2011
Orange oil 10%; gum acacia 16%; Ester gum 10%; Yellow #6 5%; Deionized water 59%	Pre-mixed for 2 min at 6000 to 7000 rpm using a high shear mixer, high pressure homogenized at 13,000 psi for one single pass	<1 μm	Reiner, S.J., Reineccius, G.A. & Peppard, T.L. 2010
Soybean oil 10%; 4% gum acacia and 1% flaxseed protein; sodium azide 0.04%; Deionized water 84.96%	Gum solution was stirred for 24 hrs, pre-mixed for 2 min at 15,000 rpm using a rotor-stator machine, high pressure homogenized at 4350, 7250, 7250, 7250 psi for four passes	<1 μm	Wang, B., Wang, L.J., Li, D., Adhikari, B. & Shi, J. 2011

Emulsion Instability Mechanisms

Emulsion stability describes the ability of an emulsion to resist changes in physicochemical properties over time (McClements, 2005). It is always important to clearly identify the dominant/major physicochemical mechanisms responsible for the instability of a particular emulsion before effective strategies can be developed to improve emulsion stability.

A variety of different physicochemical mechanisms involve in emulsion instability performance, including gravitational separation, flocculation, coalescence, Ostwald ripening and phase inversion (McClements, 2007). Gravitational separation is the process whereby droplets move upward or downward. When droplets have a lower density than the surrounding liquids, they will move upward, which is called creaming. When droplets have a higher density than the surrounding liquids, they will move downwards, which is called sedimentation. Flocculation and coalescence are two types of droplet aggregation. Flocculation occurs when two or more droplets stick together to form an aggregate in which the droplets retain their individual integrity, whereas coalescence is the process when two or more droplets merge together to form a single larger droplet (McClements, 2005). Ostwald ripening results from mass transport of dispersed phase material through the continuous phase, larger droplets grow at the expense of smaller droplets. Phase inversion is the process whereby an O/W emulsion changes into a W/O emulsion, or vice versa (McClements, 2007).

Factors Influencing Emulsion Properties

Emulsion properties are strongly influenced by droplets characteristics, including their concentration, particle size, droplet charge, interfacial properties and colloidal interactions (Dickinson, 1992; Friberg, Larsson, & Sjoblom, 2004; Qian, Decker, Xiao, & McClements, 2011; McClements, 2005 & 2007).

Droplet concentration in an emulsion plays an important role in determining its structure, stability, appearance and quality (McClements, 2005). Therefore, it is important to be able to clearly specify its droplet concentration. Droplet concentration is usually characterized in terms of dispersed phase volume fraction or dispersed phase mass fraction.

Many of the most important properties of emulsion-based food products are determined by droplet size, such as stability (gravitational separation, flocculation, coalescence, etc.), optical properties (lightness or turbidity), and rheology (viscosity or interfacial tension). Therefore, it is particularly important to be able to reliably measure droplet size, which can be reported as a radius or a diameter (McClements, 2007). Emulsions can be classified as "monodisperse" and "polydisperse". When all the droplets have the same size, the emulsion is referred as monodisperse. For a polydisperse emulsion, it is characterized as particle size distribution (PSD), in which the particle concentration is usually presented as either volume percent or number percent of droplets within a particular class (McClements, 2005). The particle size analyzer provides a full PDS of an emulsion, which contains information about the size characteristics of all of the particles presents, also providing insights into the possible origin and nature of any instability changes in the emulsion (McClements, 2007). The two most commonly used mean particle size values are the surface-weighted mean diameter ($D_{3,2}=\Sigma_i n_i d_i^3/n_i d_i^2$) and the volume-weighted mean diameter ($D_{4,3}=\Sigma_i n_i d_i^4/n_i d_i^3$). In general, the volume-weighted mean diameter is more sensitive to the presence of large particles (McClements, 2005).

The charge on a droplet is important because it determines the nature of its interactions with other charged species or its behavior in the presence of an electrical fields (McClements, 2005). In many emulsion foods, the droplets are prevented from aggregation by using ionic emulsifiers that absorb to their surface and prevent them from coming close enough because of

electrostatic repulsion (Dickinson, 1992). The properties of emulsions stabilized by ionized emulsifiers are very sensitive to pH and ionic strength of the aqueous phase. The electrical characteristics of a droplet are often characterized in terms of its surface electrical potential, surface charge density or ζ-potential. The ζ-potential is defined as the distance away from the droplet surface below which the counter ions remain firmly attached to the droplet. Among all the charge parameters, ζ-potential is much easier to measure and it accounts for the adsorption of any charged counter ions, therefore ζ-potential is broader used than others (Hunter, 1986).

The droplet interface consists of a narrow region that surrounds each emulsion droplet, which contains a mixture of oil, water and surface-active molecules (Hunter, 1989). It can have an appreciable impact on many of the most important bulk physicochemical properties of food emulsions (McClements, 2005). Therefore, it is of importance to elucidate the factors that determine the composition, structure, thickness, rheology and charge of interfacial region (McClements, 2007).

Colloidal interactions control whether emulsion droplets aggregate or remain as separate entities as well as the characteristics of any aggregates formed. There are many different kinds of colloidal interactions that may act in food emulsions, including van der Waals, electrostatic, steric, depletion, hydration and hydrophobic interactions (McClements, 2005). These individual interactions vary in (attractive or repulsive) sign, (weak to strong) magnitude, and (short to long) range. Generally, droplets tend to aggregate when attractive interactions dominate, but remain as individual entities when repulsive interactions dominate (McClements, 2005).

Surface-active hydrocolloids may act as emulsifiers and emulsion stabilizers through adsorption of protective layers at oil-water interfaces, and interactions of hydrocolloids with emulsion droplets may affect stability with respect to aggregation, e.g., gum arabic, so Dickinson

(2003) studied and summarized the main factors affecting emulsion stability (Table 2) and pointed out that for a biopolymer to be more effective in stabilizing emulsion droplet, it should exhibit four characteristics: (1) Strong adsorption, implying that the amphiphilic polymer has a substantial degree of hydrophobic character to keep it permanently anchored to the interface to lower interfacial tension dramatically. (2) Fully surface coverage, implying that there is sufficient polymer present with the ability to completely saturate the surface. (3) A thick steric stabilizing layer is formed, implying that the polymer is hydrophilic with high molecular weight and high solubility. (4) A charged stabilizing layer is formed if the polymer is not sufficiently thick. For a freshly prepared concentrated emulsion, the most obvious initial manifestation of stability is creaming, which leads on to macroscopic phase separation then into separated regions of cream and serum by clarification (Dickinson, 2003; Dickinson, 2009).

Table 2.2 Principal factors affecting oil-in-water emulsion stability in general (Dickinson, 2003)

Category	Principal Factors
Droplet-size distribution	Emulsification equipment Emulsifier concentration and type Oil to water ratio Other factors (e.g., temperature, pH, viscosity)
Nature of interfacial adsorbed layer	Emulsifier concentration and type Interactions of adsorbed species Competition between adsorbed species
Nature of continuous phase	Rheology Solvent quality and ionic environment Unadsorbed polymers and amphiphiles
Nature of dispersed oil phase	Solid/liquid content Solubility in continuous phase

Emulsion Stability Evaluation Techniques

A wide variety of powerful analytical instrumentations have been developed to characterize emulsions properties and stability of food emulsions. In general, the major emulsion properties that should be measured, with the analytical techniques for their determinations are listed as follows: (1) Emulsion microstructure, including optical microscopy and electron microscopy. (2) Particle size distribution, by using laser light scattering instrument, e.g., Malvern Master Sizer. (3) ζ-potential, by using micro-electrophoresis, e.g., Zetasizer. (4) Creaming stability, by measuring creaming index with visual observation, digital photography or centrifugation studies. (5) Rheology, by using viscometer or shear rheometer (McClements, 2007).

In previous literature, some researchers have used the above analytical techniques and instrumentation to study the stability performance of emulsions formulated with gum acacia as an emulsifier. Qian, Decker, Xiao, & McClements (2011) compared the effectiveness of β-lactoglobulin, gum acacia, conventional modified starch and new modified starch as the biopolymer emulsifiers at forming and stabilizing orange oil-in-water emulsions using particle size, ζ-potential and creaming index. They concluded that there was little effect of pH, ionic strength on emulsion stabilized by gum acacia due to strong steric stabilization, indicating that pH and ionic strength are not the major factors affecting gum acacia performance as an emulsifier.

Reiner, Reineccius, & Peppard (2010) studied the stability of beverage cloud emulsions formulated with different gum acacia and starch based emulsifiers by measuring particle size, changes in turbidity of beverage and visual observation of ringing. They found that gum acacia

and modified gum acacia emulsifiers were superior in stability to the starch-based emulsifiers at either 25°C or 35°C storage temperature for orange terpene-based beverages.

Charoen et al. (2011) investigated the effects of biopolymer emulsifier type and environmental stresses on the stability of rice bran oil-in-water emulsions, including whey protein, gum arabic and modified starch by testing particle size, ζ-potential and creaming index. They found that emulsions formed using the polysaccharide emulsifiers (gum acacia) had much better stability to environmental stresses than those formed using the globular protein emulsifier (whey protein isolate), which was attributed to differences in the nature of the colloidal interactions operating between oil droplets towards environmental stresses. Gum acacia working as an emulsifier was attributed to strong steric stabilization while whey protein isolate was attributed to changes in electrostatic and hydrophobic interactions between droplets. According to their findings, gum acacia produced large droplets and needed high concentrations, but they had good stability to stress.

Buffo, Reineccius, & Oehlert (2001) studied the factors that may affect the emulsifying and rheological properties of gum acacia in beverage emulsions, including proximal composition of the gum (protein content and mineral content), gum processing prior to emulsion preparation (pasteurization and demineralization) and pH values. They measured viscosity, particle size, turbidity, protein load, layer thickness and packing density of the gum at the interface. Their results showed that protein content was not related to emulsion stability, whereas both pasteurization and demineralization for gum processing before emulsion preparation favored stability. The possible reason was that pasteurization and demineralization could promote protein unfolding, leading to more proteins exposed outside of the molecules. They also found that beverage emulsion were less stable at pH=2.5 than at pH=4.5 or 5.5. Most interestingly,

they concluded from their study that there is a significant natural variability among *Acacia* species and even within the same species among batches from different growing regions with respect to their performance as emulsifiers. Subsequent similar processing factors aiming to enhance their emulsifying properties may have quite different effectiveness.

Gum Acacia Emulsion Storage Study

The preparation of emulsions that is kinetically stable over a time period is of practical use to the food industry, e.g., a few days, weeks, months or years. In order to characterize emulsion stability, it is needed in quality control laboratories and in food production factories to design specific storage study to monitor food emulsions and their properties from time to time for specific purpose.

Ramin, Mehranian, & Vahabzadeh (2009) designed a storage study to evaluate the effect of soy protein isolate-gum arabic composite on the stability of beverage emulsion prepared as the orange oil-in-water emulsion. They ran a long storage stability test by storing the soy protein isolate-gum acacia composite emulsion samples at 4, 16 and 30°C each for 8 days to measure the size of the emulsion droplet changes with the storage time, and found that the size of the emulsion droplet increased with the storage time.

Reiner, Reineccius, & Peppard (2010) investigated the performance of several hydrocolloids (3 gum acacias, 1modified gum acacia and 3 modified starches) in stabilizing beverage emulsions with three parameters, different core materials, emulsifier usage levels, and storage temperatures. They stored concentrated emulsions prepared with orange terpenes weighted with ester gum at 25 or 35°C, and analyzed on day 0, 1 and 3. On day 3, model beverages were made from the emulsion, stored at both temperatures, and analyzed weekly for 4 weeks by measuring mean particle size, visual observations of ringing, and loss of turbidity.

They found that emulsions stored at 35°C destabilized faster than 25 °C. They also found concentrated emulsion containing gum acacia was stable over 3 day storage at both temperatures from particle size measurements.

Conclusion

Although as a fairly expensive ingredient, gum acacia is the most commonly recognized hydrocolloid emulsifier, which is widely used in soft drink industry for emulsifying flavor oils under acidic conditions. This literature review has given an overview of gum acacia structure characteristics and emulsifying properties, as well as research techniques and outcomes of gum acacia as an emulsifier/stabilizer in previous studies. However, there are still disagreements among gum acacia structure properties, structure-function relationship and functionality, which might result from their variability in species or even within the same species from different batches. Therefore, in our work, five commercial gum acacia samples with *Acacia senegal* and *Acacia seyal*, will be structurally characterized and functionality evaluated, and a structure-function relationship will be discussed to compare their emulsifying performance.

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CHAPTER 3

PHYSICOCHEMICAL FEATURES OF ACACIA SENEGAL AND ACACIA SEYAL[1]

[1] Dong, H., Hawkins, S., and Wicker, L., To be submitted to Food Hydrocolloids.

Abstract

Commercial gum acacia (GA) from *Acacia senegal* (SG1, SG2 and SG3) and *Acacia seyal* (SY1 and SY2) were characterized by physicochemical and spectroscopic methods. The results indicated that the GAs had similar ($p>0.05$) protein values ranging from 0.80-0.90 μg/mg; ζ-potential was similar ($p>0.05$) for all GAs and was near -32 to -34 mV, but particle size varied ($p<0.05$) between 170 to 250 nm, in the order SG2<SG3>SG1 for SG samples and SY2<SY1 for SY samples. A linear regression between gum concentration and relative fluorescence intensity of an external hydrophobic probe, ANS (1-anilinonaphthalene-8-sulfonic acid), indicated higher ($p<0.05$) slope values in the order S_0 (SG2)>S_0 (SG3)>S_0 (SG1)>S_0 (SY1)>S_0 (SY2) and higher surface hydrophobicity. Raman and FT-IR spectra peaks associated with protein or carbohydrate were observed. The ratio of peak intensity between protein and carbohydrate from FT-IR was higher ($p<0.05$) for SG than SY. Therefore, ANS surface hydrophobicity, effective diameter, Raman and FT-IR may be useful tools to predict functionality of gum samples.

KEY WORDS: Surface hydrophobicity, protein, ζ-potential, Raman, FT-IR

Introduction

Gum acacia (or gum arabic) is the natural hydrocolloid obtained from the exudate of the acacia tree and is extensively used as an emulsifier/stabilizer in beverage emulsions (Tan, 2004). There are two botanical species of acacia trees approved for commercial use as sources of gum acacia: *Acacia senegal* and *Acacia seyal* (Flindt, Al-Assaf, Phillips, & Williams, 2005). It is reported that gum acacia derived from these two species differ in functionality, with *Acacia senegal* generally being considered as the industry "gold standard" emulsifier/stabilizer, which might be due to their different physicochemical or structural features (Reiner, Reineccius, & Peppard, 2010).

Numerous physicochemical studies of gum acacia have showed that it is heterogeneous and complex with mainly three distinct fractions, varying in protein content (Islam, Phillips, Sljivo, Snowden, & Williams, 1997). The major component is arabinogalactan fraction (AG), which represents about 89% of the gum and contains <1% protein, with a molecular mass of $2.5\sim3\times10^5$ Da. The second component is arabinogalactan-protein fraction (AGP), which represents about 10% of the total and contains about 10% protein, with a molecular mass of $1\sim2\times10^6$ Da. The third is glycoprotein (GP), which represents only 1% of the total but contains up to 50% protein, with a molecular mass of about 2×10^5 Da. The AGP fraction, although being a minor component, is reported to play an important role in the structure and function performance of gum acacia (Hassan, Al-Assaf, Phillips, & Williams, 2005; Al-Assaf, Phillips, & Williams, 2005; Flindt et al. 2005). "Wattle blossom " model and "twisted rope" glycoprotein model are two most popular models for AGP fraction currently in the literature (Fincher, Stone, & Clarke, 1983; Qi, Cynthia, & Derek, 1991).

Some work has been done to investigate and differentiate these two species of gum acacias structural features. Biswas, Phillips, & Biswas (1995) reported that protein content and amino acid compositions were present in the same proportions in both gum exudates from Africa. Both species of gums were composed of the same sugars (D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid and 4-O-methyl glucuronic acid) joined by the same chemical bonds in a branched structure consisting of a β-1, 3 linked D-galactose core and β-1, 6 D-galactose branch points (Siddig, Osman, Al-Assaf, Phillips, & Williams, 2005). Research work later by Biswas, Biswas, & Phillips (2000) as well as Anderson & McDougall (1987) showed that there were more arabinose and less rhamnose in *Acacia seyal* than *Acacia senegal*. Anderson & Yin (1988) indicated that the majority of amino acids in *Acacia seyal* were in deep-seated location, while the majority of amino acids in *Acacia senegal* were exposed outside and attached to sugars in peripheral positions, and there was approximately half the amount of protein in *Acacia seyal* compared with *Acacia senegal*.

The molecular weight distribution and interfacial rheology have been taken as two main aspects to distinguish *Acacia seyal* and *Acacia senegal* structural information in the previous literature. They commonly used GPC-MALLS to compare molecular weight or molecular weight distribution among different gum samples, and tried to find the correlation between gum species and molecular weight distribution (Hassan et al. 2005). Their results found extensive variations of average molecular weights both within the same gum species and between individual samples, but all were presented to the markets as "gum arabic". The interfacial rheology was first studied by Warburton (1966) and subsequently received further attention for gum acacia properties investigations (Fauconnier, et al., 2000). They found a correlation between the protein differences and the surface rheology, that *Acacia senegal* samples had

greater interfacial elasticity than *Acacia seyal* samples and increased with overall protein content and AGP fraction amount (Elmanan, Al-Assaf, Phillips, & Williams, 2008; Anderson & Yin, 1988; Buffo, Reineccius, & Oehlert, 2001).

Some work has been done in the past about the surface hydrophobicity of proteins to their conformation and functions. Kato & Nakai (1980) used *cis*-parinaric acid as a fluorescence probe and the initial slope of fluorescence intensity vs. protein concentration plot to determine the surface hydrophobicity of proteins. They observed a significant correlation for initial slope of native proteins, denatured proteins and surfactant-bond proteins, and significant correlations with interfacial tension and emulsifying activity of those proteins. Ju, Hettiarachchy, & Rath (2001) measured the surface hydrophobicity of extracted rice flour protein after heat-denaturation by using 1,8 ANS (1-anilinonaphthalene-8-sulfonic acid) as the probe, and established the relationship between protein denaturation and an increase of hydrophobicity as an efficient indicator. Fluorescence of ANS-protein interactions was also investigated in bovine serum albumin (Matulis, Baumann, Bloomfield, & Lovrien, 1999).

Therefore, in this study, we chose commercial gums as test samples, to establish the useful but simple, rapid techniques to compare these two gum acacia species for physicochemical and structural features, and to provide some information on gum products selection.

Materials and Methods

Materials

Acacia senegal samples from three lots (FCC Powder Lot No. 18508, Dry Powder-A Lot No. 19173, and Dry Powder-B Lot No. 19215), denoted SG1, SG2, SG3, and two lots of *Acacia seyal* samples (FT Powder-A Lot No. 19474 and FT Powder-B Lot No. 19398), denoted SY1 and

SY2, were a gift from TIC gums (Belcamp, MD, USA). Albumin standard (source: bovine serum albumin, fraction V, with the concentration of 2.0 mg/ml in a 0.9% aqueous NaCl solution containing sodium azide), BCA protein assay reagent A and B were purchased from Pierce, Thermo Scientific (Rockford, IL, USA). 1,8 ANS (1-anilinonaphthalene-8-sulfonic acid) was purchased from Molecular Probes (Eugene, Oregon, USA). De-ionized water was used throughout.

Analytical methods

Protein Assay

A 50 mg/ml gum dispersion in deionized water was mixed at room temperature and hydrated overnight at 4 °C. Protein was assessed using BCA assay with BSA as standard protein, and read in iMark Microplate Absorbance Reader (Bio-Rad, Hercules, CA, USA). Triplicate assays were made.

Surface Hydrophobicity

Fluorolog-2 Spectrofluorometer (SPEX industries, Inc. Edison, NJ, USA) with a xenon lamp was used to measure fluorescence due to binding of 1,8 ANS (1-anilinonaphthalene-8-sulfonic acid) to non-polar domains. A range of concentrations between 15 mg/ml and 2.5 mg/ml for each gum acacia sample solution was made in deionized water and hydrated overnight at 4 °C. A stock ANS, 1.5 mg/ml, was prepared daily in deionized water and protected from light using an aluminum foil covered container. Final concentration of ANS in 2 ml of gum solution was 1.5 μg/ml (5 μM) and relative fluorescence was recorded after three minutes. Excitation and emission monochrometers were set at 380 nm and 475 nm, respectively. Excitation and emission slits were set at 1.88 nm and 9.42 nm band pass, respectively. Preliminary trials indicated that these conditions gave a high signal to noise ratio, above

background counts. Relative fluorescence intensity is reported after subtraction of background of ANS in water and relative to ANS fluorescence in ethanol to account for daily lamp fluctuations. Surface hydrophobicity was calculated based on the following equation:

$(F-F_0)/F_0=K\Delta C$ (Qu, Lu, Yan, Zhou, & Zuhong, 2009)

F and F_0 are the ANS fluorescence intensity in the presence and the absence of the samples; ΔC is the concentration of the samples; K is the slope, representing the equilibrium constants.

Particle Size Analysis

The particle size was determined with a 90 Plus Particle Size Analyzer (Brookhaven Inc., Holtsville, NY). A 0.5 mg/ml gum solution in 10 mM potassium nitrate buffer was mixed and sonicated at room temperature for 30 s for full dissolution (Instruction manual for 90Plus, 1995). The solution was filtered with a 1 ml Monoject Turberculin syringe without needle (Tyco Healthcare Group LP, Mansfield, MA, USA) connected with a 5 µm hydrophilic polyvinylidene (PVDF) filter (Millipore Corporation, Billerica, MA), and then pipetted about 3 ml into the cuvette. All filters, syringes, pipettes and cuvettes were passed through/rinsed with filtered 10 mM potassium nitrate buffer before use. Sample preparation was conducted under a stainless steel biological control laboratory hood (Contamination Control Inc., Kulpsville, PA) to minimize dust contamination. Measurements were taken after 5 minutes to ensure temperature equilibration. Each sample was tested in triplicate for five measurements each. The temperature was set at 25°C and dust cutoff value was set as 30 with sample quality (a number shown on the screen indicating of the difference between the measured and calculated baseline of the correlation function) above 9.

ζ-Potential

The charge on the surface of particles was analyzed by measuring the ζ-potential. This measurement was obtained by using a Particle Size Analyzer adding the BI-Zeta option (90 Plus, Brookhaven Inst., Holtsville, NY) with a 50 mV diode laser (90° angle) and a BI-9000AT correlator. A 0.5 mg/ml gum sample in 10 mM potassium nitrate buffer was mixed. The electrode for surface charge measurement was rinsed with filtered 10 mM potassium nitrate buffter before use. Gum samples were tested at pH 5.5 for 10 runs and in triplicate analysis.

Fourier transform infrared-attenuated total reflection (FTIR-ATR) spectroscopy

FT-IR spectra of each gum sample was obtained using a Nicolet Magna 850 FT-IR bench (Thermo Nicolet, Madison, WI) employing a Golden-gate Diamond single internal reflection element (IRE) DuraScope ATR sampling device (Smiths Detection, Danbury, CT) equipped with a deuterated triglycine sulfate (DTGS) detector. A small sample of gum powder was placed on top of an ATR plate. Contact pressure level 5 on the electronic load display scale was employed to apply constant pressure between samples, by way of a stainless steel rod. The spectra was collected at the absorbance wavenumber ranging from 4000 to 500 cm^{-1} at 8 cm^{-1} resolution with 64 co-added scans. Each sample was tested in triplicate.

Raman spectroscopy

Raman spectra of each gum sample were obtained by using a SENTINEL Raman Process Spectrometer (Bruker, Billerica, MA, USA), with an integrated diode laser of 785 nm wavelength. Power level of 400 mW was applied. Acetaminophen was used for calibration. A small portion of gum sample powder was put into a glass cuvette for a measurement. Each sample was tested in triplicate. A manual baseline correction was used when analyzing the spectra.

Statistical analysis

Data results from triplicate sample preparation and at least triplicate assays. Protein content, particle size, polydispersity index and slopes of surface hydrophobicity of 5 types of gum acacia samples were statistically analyzed using one-way ANOVA at a significance level of 5%. Statistical analysis was done using Microsoft Excel and Statplus:Mac. Raman and FT-IR spectra were analyzed using OPUS software and OMNIC software. Representative spectra are presented. Peak ratio calculations result from triplicate spectra.

Results and Discussion

Physicochemical characteristics of gum samples were tested. It can be seen from the data in Table 3.1 that protein contents of 5 different types of gum acacia samples were in the range of 0.85-0.90 μg/mg (weight basis). There were no significant statistical differences in protein content among the five lots of gum samples ($p>0.05$), indicating that total protein content is not likely to be a reliable factor to evaluate the functionality. The protein content was lower than that previous reported by Al-Assaf et al. (2005) with 1.8-2.1 μg/mg for *Acacia senegal* samples, and Hassan et al. (2005) with 0.7-1.3 μg/mg for *Acacia seyal* samples using nitrogen analysis. These differences might be partially due to that BCA protein assay measured soluble protein only, also according to Hassan et al. (2005) and Al-Assaf et al. (2005), the variations of protein content exist, which might be attributed to natural gum trees derived from different geographical locations and soils or different gum products processing methods applied or blending with other gum samples. Here, therefore, the possibly reliable indicator to distinguish protein differences between *Acacia senegal* and *Acacia seyal* might be protein distribution in molecular weight but not the total protein content, which was reported by Findt et al (2005) in GPC profile and Randall, Phillips, & Williams (1988) in RI and UV elution profiles.

The effective diameter represents an average size of the particles in the gum aqueous system (Instruction manual for 90Plus, 1995). The data (Table 3.1) showed that 5 types of gum acacia samples were in the range of 172-257 nm. For SG specie, effective diameter was significantly different ($p<0.05$) and the order was SG2 (172+3 nm) < SG3 (196±2 nm) <SG1 (257±5 nm). For SY specie, it was in the order that SY2 (178±6 nm) <SY1 (244±2 nm). However, particle size was not a distinguishing characteristic of gum species, as effective diameter of SG2 and SY2 or SG1 and SY1 was not different ($p>0.05$).

The ζ-potential of all gum acacia samples were negatively charged, between -32 and -34 mV. The charge is most likely attributed to the presence of some negatively charged protein side groups, especially COO^-, on these polysaccharide molecules (Padala, Phillips, & Williams, 2009). There were no significant differences ($p>0.05$) in the 5 gum acacia samples.

For each of the 5 gum solutions, the fluorescence relative intensity ($(F-F_0)/F_0$) in the presence of ANS increased linearly with an increase in gum concentrations in the range of 0-15 mg/ml, with a high correlation coefficient (R^2) above 0.92 (Figure 3.1). But they generated significantly different slope values for different gum samples, S_0 (SG1)=0.221, S_0 (SG2)=0.368, S_0 (SG3)=0.325, S_0 (SY1)=0.218, S_0 (SY2)=0.151, with the order S_0 (SG2)>S_0 (SG3)>S_0 (SG1)>S_0 (SY1)>S_0 (SY2), showing S_0 (SG)>S_0 (SY). ANS is non-fluorescent in polar environments and as a fluorescent probe. ANS can track hydrophobic side chains of proteins by binding them, which is reported as ANS fluorescence intensity (Gasymov & Glasgow, 2007). Lee (2006) used ANS to monitor whey protein and pectin interactions with ANS and reported that sugar beet pectin had higher surface hydrophobicity than citrus pectin. Kato & Nakai (1980) reported that the slope of fluorescence intensity vs. protein concentration predicted protein hydrophobicity and directly correlated with protein functionality. The slopes of *Acacia senegal*

samples were higher than the slopes of *Acacia seyal* samples, indicating higher surface hydrophobicity in *Acacia senegal* than *Acacia seyal*. The protein content showed no significant difference between these two gum species (Table 3.1), so the slope differences likely result from greater exposure of hydrophobic amino acid in *Acacia senegal* than in *Acacia seyal*, as previously suggested (Anderson & Yin, 1988; Ju et al. 2001).

As a qualitative measurement, Raman spectra 5 different types of gum acacia samples are shown in Figure 3.2. There were no intense peaks in the range of 1670-1640 cm^{-1} for all gum samples, which is the amide I region (O=C-OH) for proteins (Chourpa, Ducel, Richard, Dubois, & Boury, 2006), suggesting protein content in each gum sample is very low, consistent with previous protein assay result. The peak in the wavenumber range of 1100-1070 cm^{-1} is the amide III region, corresponding to the bonds N-H (Vandenabeele et al., 2000). The rest of the spectra are dominated by vibrational bands of polysaccharide moieties. The range of 1480-1430 cm^{-1} and 1400-1300 cm^{-1} are CH_2 and CH_3 bands. The peak intensity of SG3 was the highest, and next was SG1, the last were SG2, SY1 and SY2. Another important wavenumber range is in 950-800 cm^{-1} that is glycosidic ring stretching of sugars (O=C-O), which can be used to distinguish SG3 from the other gum samples, with the highest peak. Therefore, the food industry might apply Raman spectroscopic technique to test their commercial gum products for functional groups.

FT-IR spectra of 5 different types of gum samples are shown in Figure 3.3. The major functional groups present in all gum samples were 3550-3230 cm^{-1} (O-H stretching), 2900-2800 cm^{-1} (C-H stretching), 1630-1600 cm^{-1} and 1400 cm^{-1} (O=C- stretching), 1200-900 cm^{-1} (finger-print of carbohydrates, -O-C- stretching), and 830-500 cm^{-1} (O=C-O) (Cui, Nikiforuk, Blackwell, & Phillips, 2007). The broad peak in the range of 3550-3230 cm^{-1} represents the intermolecular

hydrogen bonded O-H (Socrates, 2001). The peak intensity of alkane group residues (C-H) region in 2900-2800 cm^{-1} was similar for all gum samples. The narrow but weak peak in 1630-1600 cm^{-1} and 1400 cm^{-1} for O=C- bond, associated with amide (proteins), suggesting low protein content in all gum samples, which is consistent with previous protein assay result and Raman spectra. The very strong peak in 1200-900 cm^{-1} is the fingerprint of carbohydrate, -O-C- stretching (Engelsen & Nørgaard, 1996), indicating a large moiety of polysaccharide in gum acacia.

The FT-IR peak intensity ratios in three regions representing the ratios of protein/carbohydrates, with peak 1 (1600-1601 cm^{-1}), peak 2 (1410-1412 cm^{-1}) and peak 3 (1020-1024 cm^{-1}), are shown in Table 3.2. From the data, the SG samples presented lower numbers of both peak1 to peak 3 ratio and peak 2 to peak 3 ratio than SY samples, indicated higher protein to carbohydrate in SG.

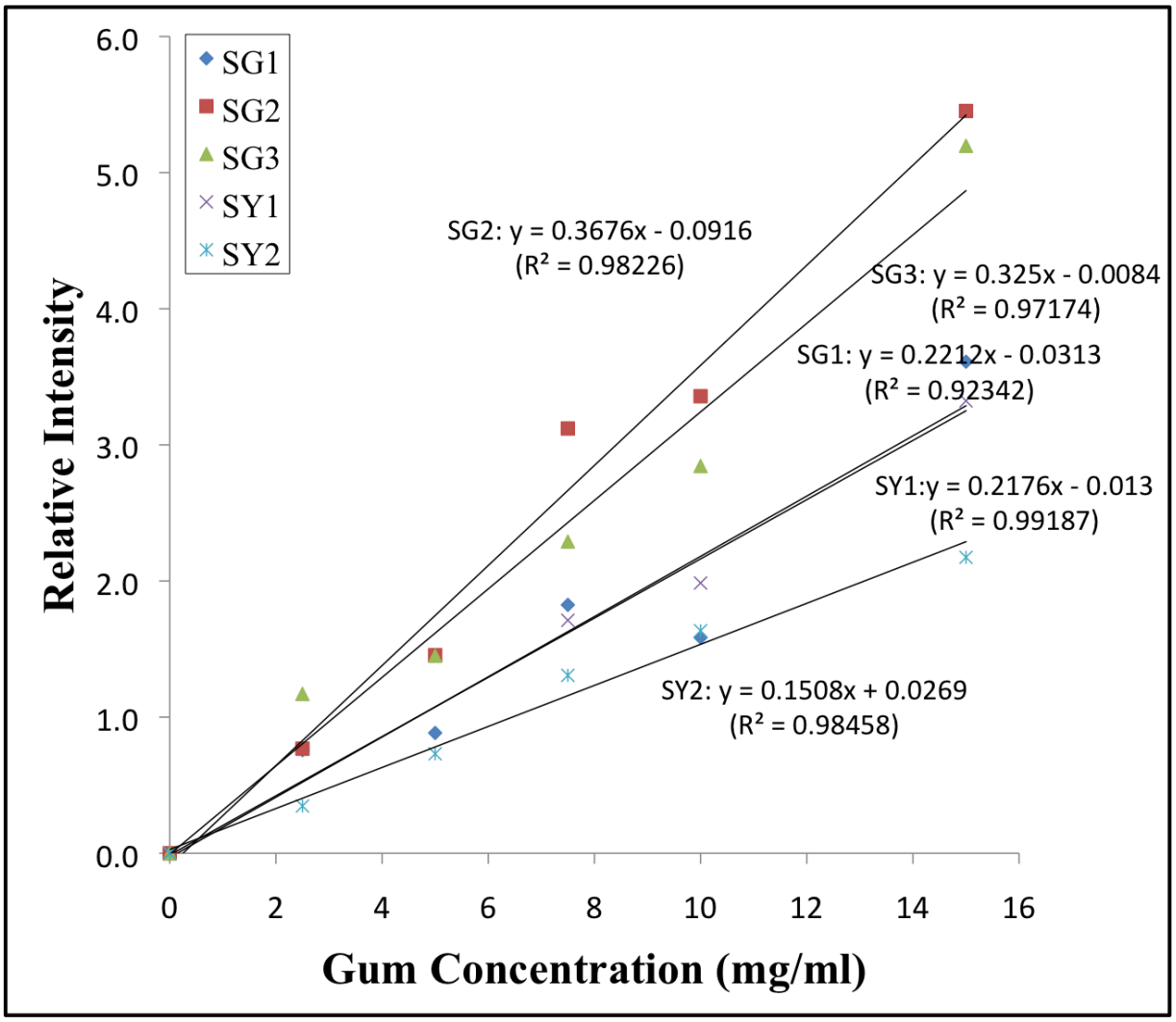
Conclusions

In this study, physicochemical methods, fluorescence probes and spectroscopic techniques were used to characterize the structures of five commercial gum samples from *Acacia senegal* and *Acacia seyal* species. Protein content and ζ-potential charge were not effective parameters to distinct the gums or between gum species. Effective diameter, surface hydrophobicity, and FT-IR protein to carbohydrate peak ratios were different between the gums, but were not directly related to gum species. Surface hydrophobicity and protein/carbohydrate ratio were effective tools to distinguish gums and gum species. These variables provide additional information about the structure of gum acacia that are likely to be useful in predicting functional attributes.

Table 3.1 SG and SY gum samples physicochemical characteristics [a]

Gum species	Gum sample	Protein content (μg/mg)	ζ-potential (mV)	Effective diameter (nm)
Acacia senegal	SG1	0.90[a]±0.02	-34[a]±1	257[a]±5
	SG2	0.89[a]±0.02	-32[a]±1	172[c]±3
	SG3	0.90[a]±0.04	-32[a]±0	196[b]±2
Acacia seyal	SY1	0.85[a]±0.01	-34[a]±1	244[a]±2
	SY2	0.90[a]±0.02	-32[a]±1	178[c]±6

[a] All values are presented as mean ± standard deviation of nine measurements from triplicate extractions.
[a] Within a column for all gum samples, if $p<0.05$, sample are significantly difference



S_0 was significantly different ($p<0.05$) for all gum acacia products

Figure 3.1 Gum acacia sample surface hydrophobicity-concentration relationship

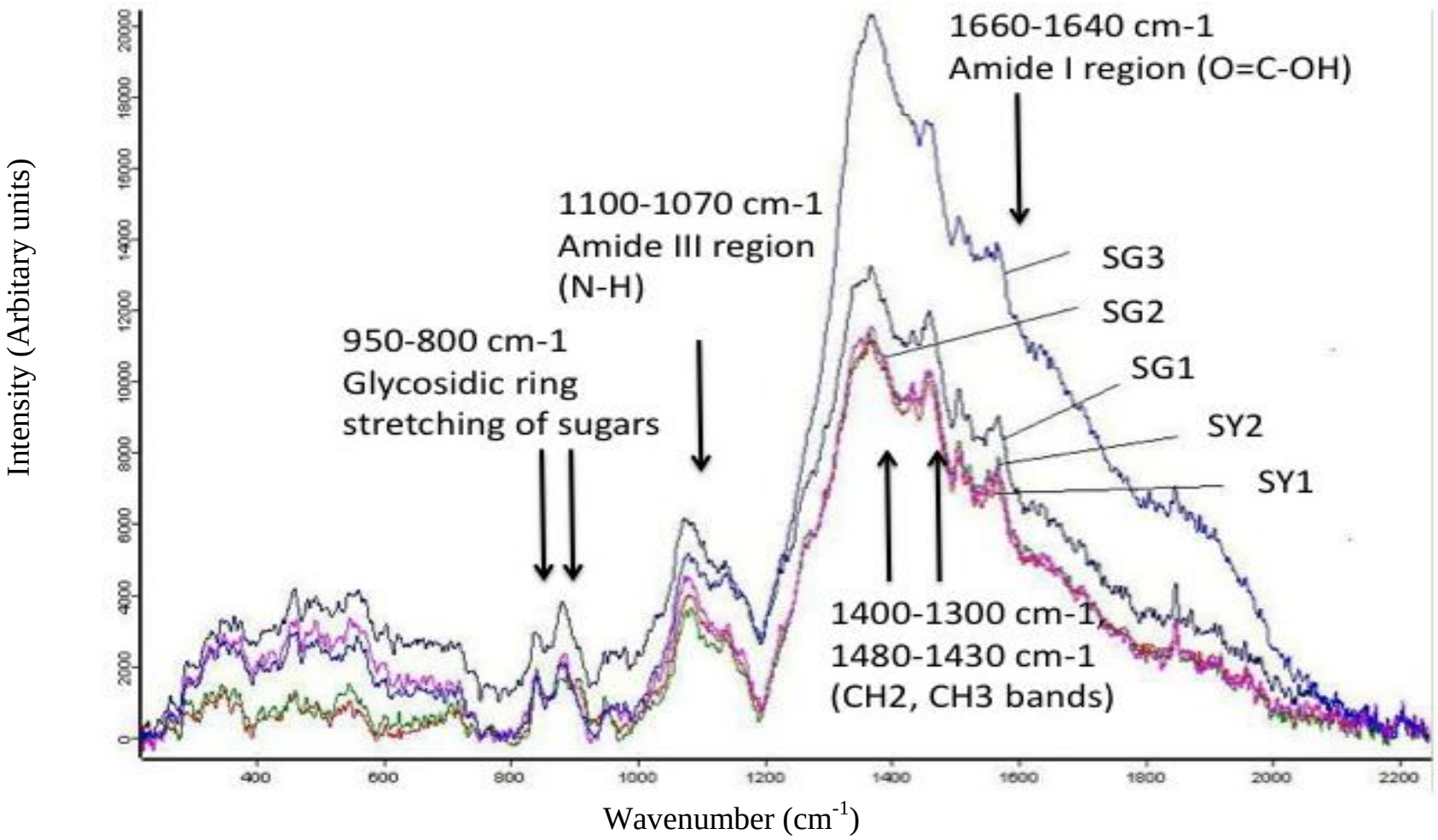


Figure 3.2 Representative Raman spectra of the wavenumber region 200-2200 cm^{-1} of 5 different types of gum acacia sample: SG1, SG2, SG3, SY1 and SY2

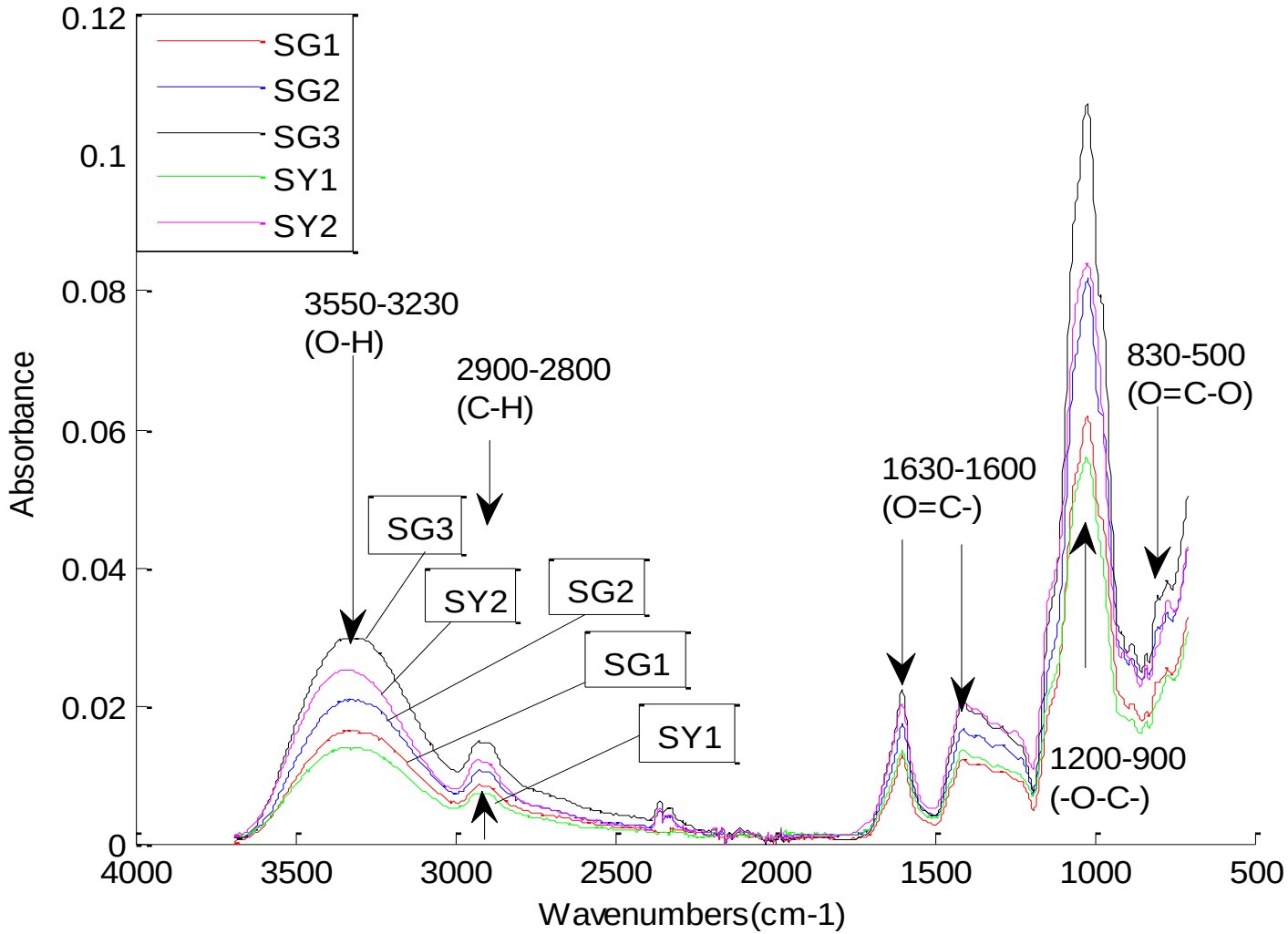


Figure 3.3 Representative FT-IR spectra of the wavenumber region 500-3800 cm^{-1} of 5 different types of gum acacia sample: SG1, SG2, SG3, SY1 and SY2

Table 3.2 FT-IR peak intensity ratios of protein/carbohydrates in all gum samples [a]

Gum sample	Peak 1 (1600-1601) Intensity	Peak 2 (1410-1412) Intensity	Peak 3 (1020-1024) Intensity	Peak 1/peak 3 ratio	Peak 2/peak 3 ratio
SG1	0.0139	0.0134	0.0671	0.21	0.20
SG2	0.0180	0.0169	0.0860	0.20	0.20
SG3	0.0229	0.0215	0.1055	0.22	0.20
SY1	0.0182	0.0182	0.0767	0.24	0.24
SY2	0.0187	0.0193	0.0762	0.25	0.25

[a] All peak intensity values are presented as the mean of three measurements

[a] Peak ratios showed no significantly different for the same gum acacia species, but were significantly different ($p<0.05$) for different gum acacia species.

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CHAPTER 4

ORANGE OIL-IN-WATER EMULSION STABILITY FORMULATED WITH ACACIA SENEGAL AND ACACIA SEYAL[2]

[2] Dong, H and Wicker, L., To be submitted to Food Hydrocolloids.

Abstract

Commercial gum acacia (GA) from *Acacia senegal* samples (denoted as SG1, SG2 and SG3) and *Acacia seyal* samples (denoted as SY1 and SY2) were used as emulsifiers in orange oil model emulsions. Emulsion stability performance was evaluated by back scatter (BS), particle size distribution and microscopic observation in a 6-day storage study at 4, 25 and 40°C. Emulsion destabilization occurred with particle migration (creaming and sedimentation), particle size increase (coalescence) and particle aggregation (flocculation), and the destabilization rate increased as storage temperature increased. Creaming of SY but not SG stabilized emulsions was observed at day 1 at 25 °C and 40°C. After subtraction from day 0, sedimentation rates showed that SG2 was more stable than other SG stabilized emulsions. Particle size distribution of SG stabilized emulsions increased between day 1 and 2, but were still monodisperse and less than 10 μm. Particle size distribution of SY stabilized emulsions was initially polydisperse and volume based distribution increased to particle sizes near 100 μm.

KEY WORDS: PSD, particle migration, coalescence, creaming

Introduction

Oil-in-water (O/W) emulsions are commonly used in the food beverage industry to impart opacity or flavor (Tan, 1997; Reineccius, 2006). Emulsions are thermodynamically unstable by nature because the contact between oil droplets and water molecules is unfavorable. Thus, emulsion stability is an important quality criterion in citrus drinks and beverages. Gum acacia, a mixture of anionic polysaccharides and protein fractions, possessing emulsifying as well as stabilizing abilities, is well known and widely used as both an emulsifier and stabilizer in the food industry (Castellani, et al., 2010). The basic principle is that gum acacia can form a protective coating around the oil droplets that prevents the droplets from aggregating with one another primarily through steric interactions (Dickinson, 2003).

There are two main botanical gum species, *Acacia senegal* and *Acacia seyal*, both originated from Sudan and are processed into commercial gum products. Generally, commercial products processed from *Acacia senegal* specie is known as the "gold standard" emulsifier in the food industry (Randall, Phillips, & Williams, 1988; Reiner, Reineccius, & Peppard, 2010). A lot of research work has been reported trying to compare these two species from either structural properties or functional properties, or a combination of both (Siddig, Osman, Al-Assaf, Phillips, & Williams, 2005; Su, Flanagan, & Singh, 2008; Biswas, Biswas, & Phillips, 2000). In addition, there are several commercial gum products with the same specie probably based on different composition or processing methods with certain advantageous functionality, suitably applied in specific food products.

The particle size and size distribution of oil droplets play a predominant role in emulsion stability (McClements, 2005). In an emulsion, the stability is a measure of the rate at which an emulsion creams, flocculates, clarifies or coalesces. The emulsion stability for gravitational

separation can be enhanced by decreasing the droplet size. Generally as a rule, larger oil droplets tend to coalesce faster than the smaller ones. Stoke's law states that the velocity at which a droplet moves is proportional to the square of the droplet size radius. A decrease in its average droplet diameter by a factor of two may decrease the coalescence rate by a factor of 100 (Bergenstahl & Claesson, 1990).

Most of the recent work on gum acacia as a food-grade emulsifier and stabilizer in oil-in-water emulsions involved the comparison of gum acacia and other types of emulsifiers, i.e. modified starch, whey protein, soybean protein (Reiner, Reineccius, & Peppard, 2010; Charoen, et al., 2011; Wang, Wang, Li, Adhikari, & Shi, 2011); a mixture of gum acacia and other types of emulsifiers, i.e. xanthan gum (Desplanques, Renou, Grisel, & Malhiac, 2012; Mirhosseini, Tan, Hamid, & Yusof, 2008), sodium caseinate (Ye, Edwards, Gilliland, Jameson, & Singh, et al., 2012); conventional gum acacia and modified gum acacia, i.e. SUPER GUM^{TM} (Tipvarakarnkoon, Einhorn-Stoll, & Senge, 2010; Castellani, et al., 2010). In addition, a variety of analytical methods have been developed to evaluate emulsion stability, including but not limited to particle size distribution, ζ-potential, creaming index and rheology (McClements, 2007).

In a recent study, Dong, Hawkins, & Wicker (2012) reported that effective diameter, surface hydrophobicity and FTIR spectra were different in five commercial gum acacia samples. The objective of the current study was to prepare emulsions with cold pressed orange oil and characterize the stability and de-stabilization kinetics of emulsions.

Materials and Methods

Materials

Orange oil (batch No. 1000571125) was a gift from Firmenich (Princeton, NJ, USA). Gums tested included three lots of *Acacia senegal* samples (FCC Powder Lot No. 18508, Dry Powder-A Lot No. 19173, and Dry Powder-B Lot No. 19215) donated as SG1, SG2, SG3, and two lots of *Acacia seyal* (FT Powder-A Lot No. 19474 and FT Powder-B Lot No. 19398) donated as SY1, SY2; gums were a gift from TIC Gums (Belcamp, MD, USA). De-ionized water was used throughout. Sodium benzoate was analytical grade (Avantor Performance Materials, Inc., Phillipsburg, NJ, USA).

Oil in water emulsion preparation

Emulsion concentrates were prepared in a modified formula according to Buffo, Reineccius, & Oehlert (2001): gum acacia 18.0%, orange oil 6.0% (with a ratio of gum acacia: orange oil= 3:1), preservative (sodium benzoate) 0.1%, and de-ionized water 75.9%.

An aliquot of 18 g of each gum sample and 0.1 g sodium benzoate was slowly added to 75.9 ml de-ionized water, while stirring at room temperature until fully dissolved. Dispersions were equilibrated at 4 °C for 24 hr for full hydration. An aliquot of 6 g orange oil was slowly added to the gum solution and gently shaken to mix. Coarse emulsion premixes were pre-homogenized 5 min on ice with a PRO300A homogenizer (Pro Scientific Inc., Oxford, CT) connected to a 5mm generator. Pre-emulsions were homogenized at 34.5 Mpa (Emulsi-Flex C5, Avestin Inc, Ottawa, ON, CA). A total of three passes were made and emulsions were kept on ice between passes. The pH of all emulsions was between 5.1-5.4. Emulsions were distributed into flat tubes for Turbiscan analysis or stored in beakers covered with aluminum foil for PSD and microscopy testing. The samples were stored for 6 days at three locations with different

temperatures, walk-in cool room set at 4°C, isotemp lab incubator set at 25°C with temperature uniformity ±0.7°C (Thermo Fisher Scientific Inc., Houston, TX) and VWR model 2020 lab incubator set at 40°C with temperature uniformity ±0.5°C (VWR International, Radnor, PA).

Analytical Methods

Emulsion stability

The TurbiSoft Classic MA2000 (Formulaction, Toulouse, France) was used to evaluate the emulsion stability in three zones over time. An aliquot of 7 ml emulsion was transferred to a flat bottom notch cap test tube and the backscattering (BS) was collected at room temperature at indicated time intervals. Samples were removed from incubator and immediately tested before replacement at storage temperature. Each sample was measured in duplicate from triplicate emulsions.

The destabilization kinetic for the emulsion clarification at the top of the tube was calculated at -10% on the corrected BS plot after subtraction from day 0 based on a modified method from Mengual, Meunier, Cayre, Puech, & Snabre (1999) to determine the settling rate (Rivner, PhD. Dissertation, 2008). The 10% corrected BS on the y-axis represented an area in the tube where BS could be measured at all time points; and the x-axis was the corresponding elapsed time.

Particle Size Distribution (PSD)

Particle size distribution was determined at room temperature with a Malvern Mastersizer (Model MSS, Malvern Instruments Ltd, Worcestershire, UK). Particle size distribution were calculated and the surface-weighted mean diameter and the volume-weighted mean diameter were expressed as $D_{3,2}$ and $D_{4,3}$, respectively. $D_{3,2}=\Sigma_i n_i d_i^3/n_i d_i^2$ and $D_{4,3}=\Sigma_i n_i d_i^4/n_i d_i^3$,where n_i is

the number of particles with the diameter of d_i. The sample beaker was shaken before testing. Each sample was tested in duplicate from triplicate emulsions.

Microscopic observation

Leica DMLB light microscopy (Leica Microsystems Inc. Buffalo Grove, IL) was used to visually observe and to image the sizes and shapes of particulates in the emulsions. Optics of 100×-enlargement lens was applied. Images were analyzed by SPOT version 4.7 software. Each sample was tested in duplicate for three replicate emulsions.

Results and Discussion

The emulsion stability during storage at 40°C (Figure 4.1) and 25°C (Figure 4.2) was analyzed on a daily basis by TURBISCAN. Selected TS graphs for SG2 (Figure 4.1.a; 4.2.a.) and SY1 (Figure 4.1b; 4.2.b) are presented. The x-axis refers to the emulsion tube length from 0 mm to 60 mm, with 0 mm being the bottom of the tube and 60 mm being the top. The y-axis refers to backscattering (BS). The different series correspond to the time at which each sample was read, and the units are hours: minutes: seconds.

The BS profiles of all emulsions showed a decrease (begins from the measure point starting to change) at the bottom of the tube (1.5-10 mm), a decrease (ends at the measure point in a merge) at the middle (10-50 mm) and an increase at the top (50-55mm), which represents sedimentation at the bottom, particle size increase in the middle and creaming at the top, respectively (Garg, Martini, Britt, & Walsh, 2010; Mengual, Meunier, Cayre, Puech, & Snabre, 1999). TS results indicated a destabilization process occurring in all emulsion samples in the 6 days storage period at 40°C and 25°C, while BS signal had little change at 4°C in preliminary trials (data not shown). The BS signal change is directly dependent on the particle mean diameter (Mao, Yang, Xu, Yuan, & Gao, 2010). In addition, from Figure 4.1 and Figure 4.2,

creaming is evident in SY stabilized emulsions at the first day that is higher at 40°C than at 25°C. Meanwhile at day 1, SG stabilized emulsions at 25 °C, had little creaming , although more creaming was observed in SG stabilized emulsions stored at 40 °C than at 25°C.

From microscopic observation of emulsions at day 0 (Figure 4.3a), differences in size and dispersity are observed between emulsions stabilized by the different gums. After storage at 4°C for 2 days (Figure 4.3b), there is clear evidence of aggregation and there are differences in aggregation in emulsion stabilized by the different gums.

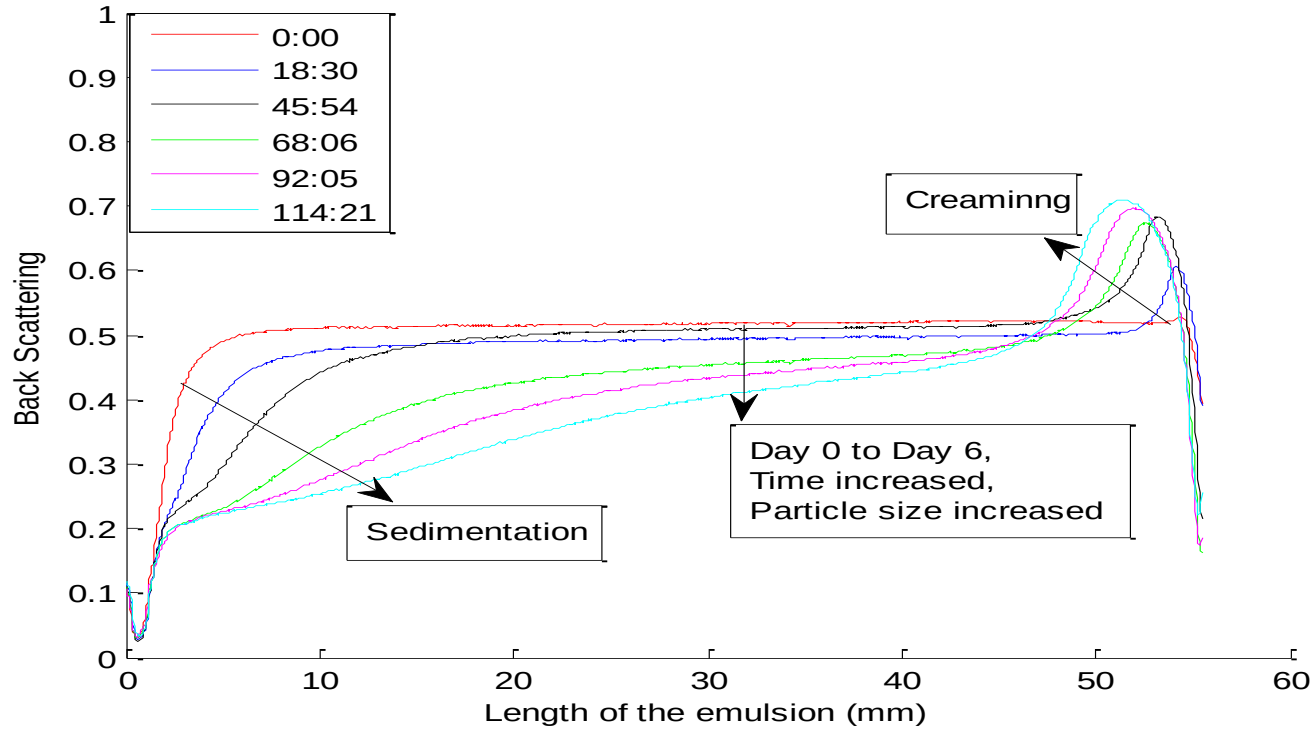
The sedimentation rates, estimated at 10% BS of SG1stabilized emulsions of all replications stored at 25°C and 40°C, are depicted in Figure 4.4. The slope and correlation coefficient of sedimentation rates of all gum stabilized emulsions are summarized in Table 4.1. Sedimentation distance increased over time in the 6 days storage period in all emulsions. The R^2 is greater than 0.92 in all cases, indicating good correlation between time and sedimentation rate. The higher slope (m) values indicate higher sedimentation rates and were compared to analyze the effects of storage temperatures and different gum species on stability of emulsions. The slope values were higher at 40°C than 25°C, indicating higher temperature facilitated emulsion destabilization. The slope values for emulsions stabilized by different gums were significantly different ($p<0.05$) in the order as m (SG2)<m (SG3)<m (SG1). This suggests that emulsion stabilizing ability of gums is in the order of SG2>SG3>SG1. However, the slope of sedimentation rates by time for emulsions stabilized by SY1 and SY2 showed no significant difference. The appearance of the emulsions at day 0 and after six days storage at 40°C or 25°C are depicted in Figure 4.5 a, b, and c, respectively. Arrows denote changes at the bottom and top of the tube to correspond with Turbiscan results.

Representative results of PSD of emulsions stabilized by SG1 and stored at different temperatures are shown as volume distribution vs particle size in Figure 4.6 a, b, and c. Representative plots of SY1 stabilized emulsions are depicted in Figure 4.7 a,b, and c. Malvern $D_{4,3}$ and $D_{3,2}$ values for all gum samples stored at 4, 25 and 40°C are shown in Table 4.2. SG stabilized emulsions were more monodisperse at all temperatures and storage times than SY stabilized emulsions. At day 1, $D_{4,3}$ values were less than 2.2 μm at all temperatures and increased to less than 3.3 μm at all temperatures or times of storage. The $D_{4,3}$ and $D_{3,2}$ values increased with temperature. In SY stabilized emulsions, the $D_{3,2}$ values remained relatively constant although the volume distribution decreased. Notably, the $D_{4,3}$ values doubled or tripled in emulsions stored at 4°C or 25°C, but decreased in emulsions stored at 40°C. The latter is thought to be due to sampling error, based on the visual appearance (Figure 4.5.b). Emulsions were polydisperse with emulsion droplets within a range below 10 μm or below 100 μm (SY1 and SY2 emulsions stored at 25 °C and 40 °C). When stored at 4 °C, emulsion PSD had a big shift in the later days compared to the first day, and a small shift within the following 2^{nd} to 6^{th} day (data not shown). As storage days increased, the volume of smaller particle sizes below 4 μm decreased, and the volume of larger particle sizes above 4 μm increased. These results indicated that as storage temperature increased, the particle size increased, but the nature of PSD changes was different. For SY stabilized emulsions, there were shifts towards the volume distribution (up and down), but little shift about the particle size changes (left and right). The appreciable difference between the values of $D_{4,3}$ and $D_{3,2}$ for SY samples indicating that the PSD was broader, while SG samples PSD was narrower (McClements, 2007). Notably, the trends in changes in emulsion stability and particle size agreed with higher surface

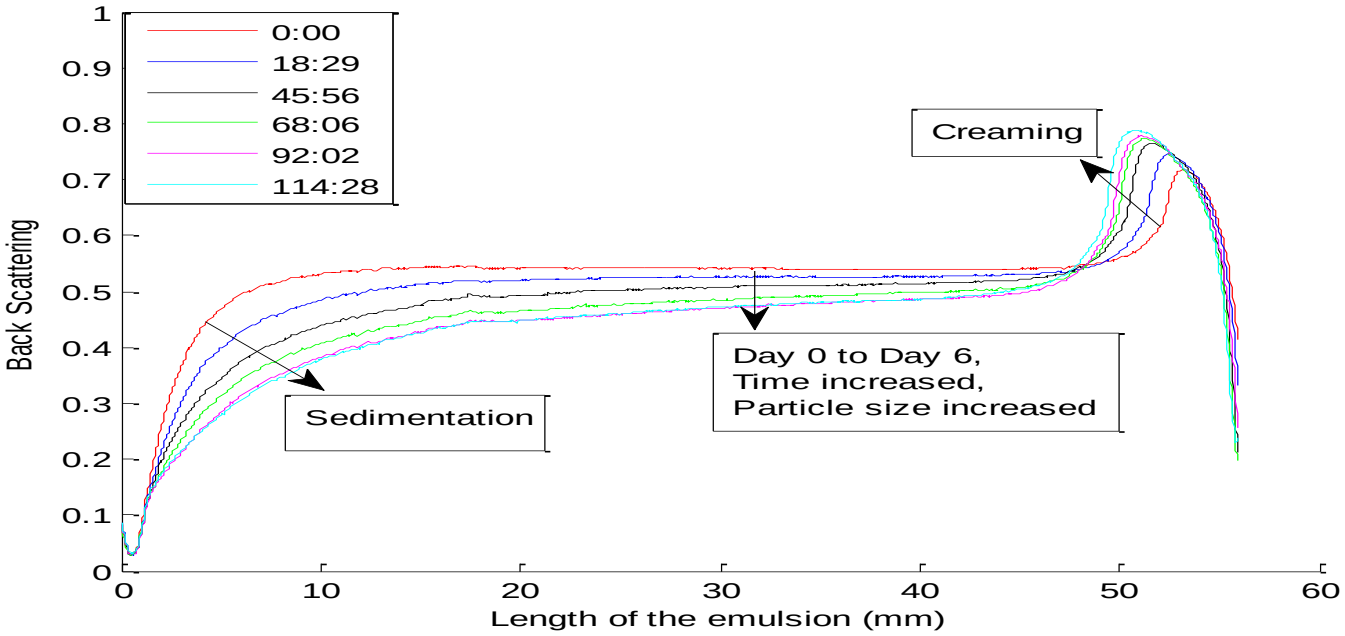
hydrophobicity, small effective diameter, and higher protein to carbohydrate ratio in physical properties of the gums (Dong, H., Hawkins, S. & Wicker, L., in preparation).

Conclusions

In this study, the effects of gum species and storage temperature on the stability performance were studied. The use of TURBISCAN to test emulsion destabilization process combined with Malvern to measure particle size distribution are easy but effective way to evaluate stability performance in an emulsion ageing study. Overall, SG emulsion was more stable than SY emulsion, and SG2 works better than other SG samples. As storage temperature increased, the emulsions tended to destabilize faster, but SG samples and SY samples behaved slightly different. SG samples PSD had a shift towards particle sizes, but still as one peak. SY samples PSD had a shift towards the volume distribution, and they had larger particles within 100 μm, as two peaks.

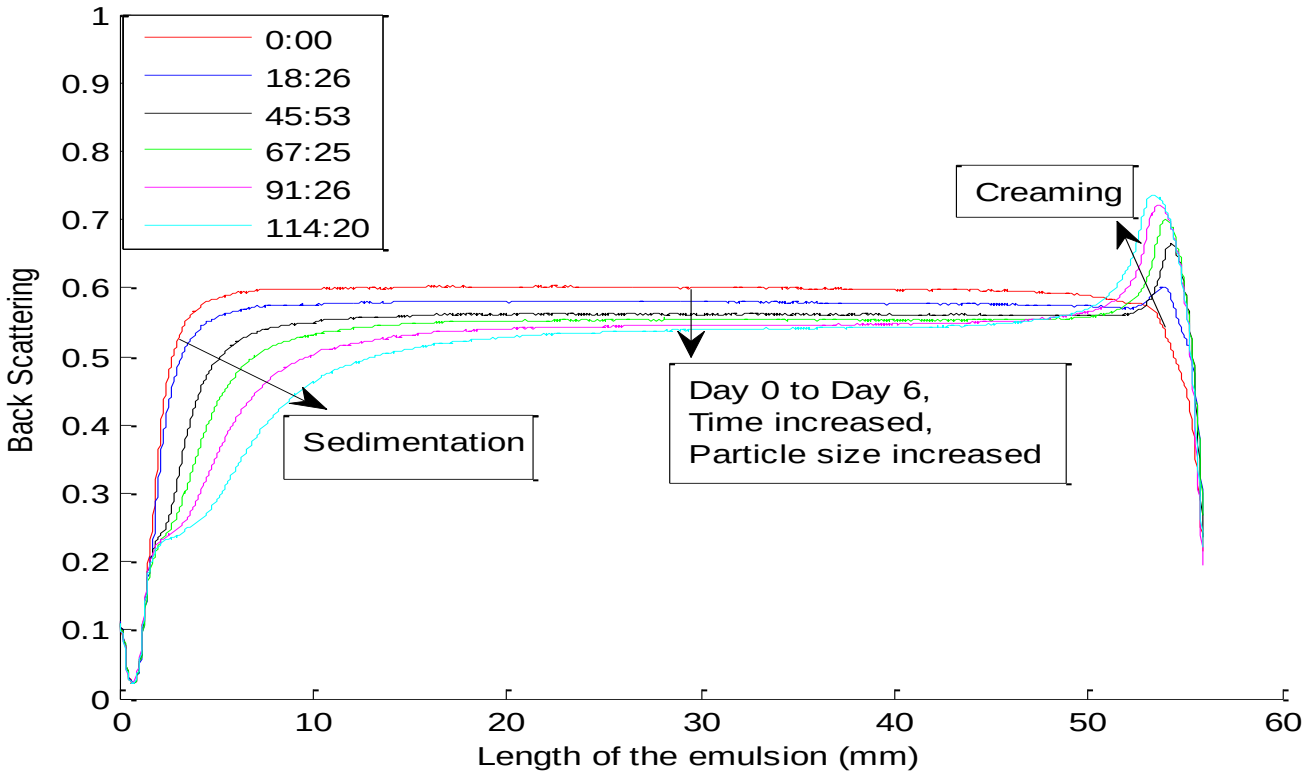


4.1-a

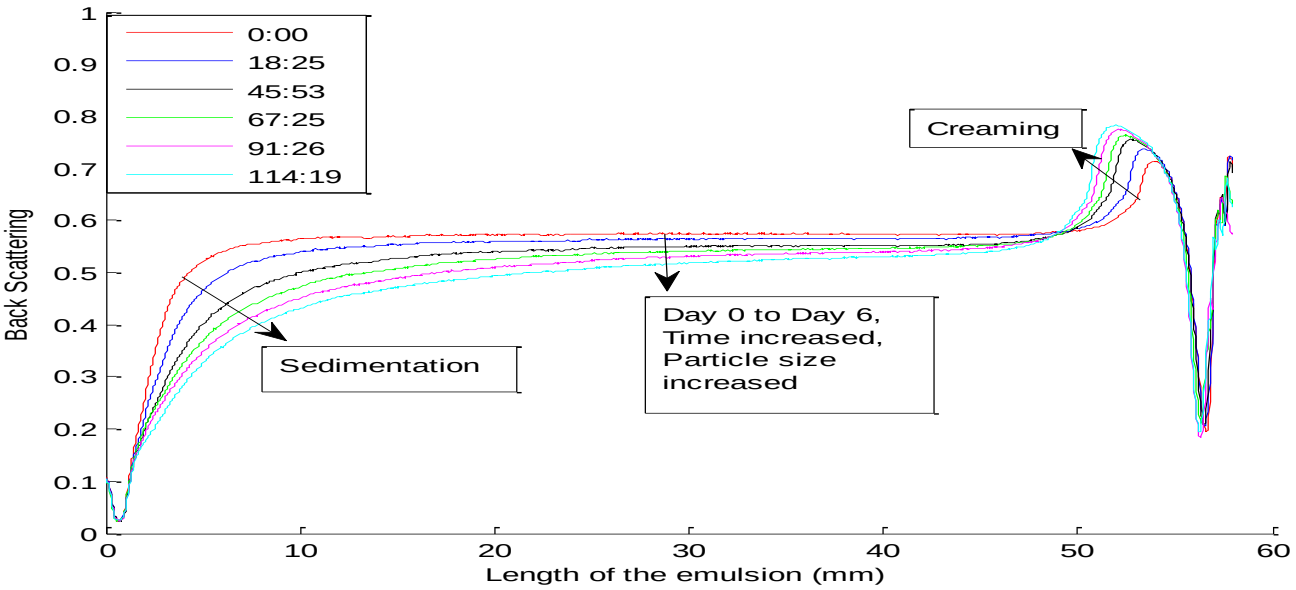


4.1-b

Figure 4.1 Turbiscan back scattering profiles formulated with representative SG and SY @ 40°C (4.1-a: SG2; 4.1-b: SY1). These data are represented as a function of time (hr: min) and of sample height (mm).

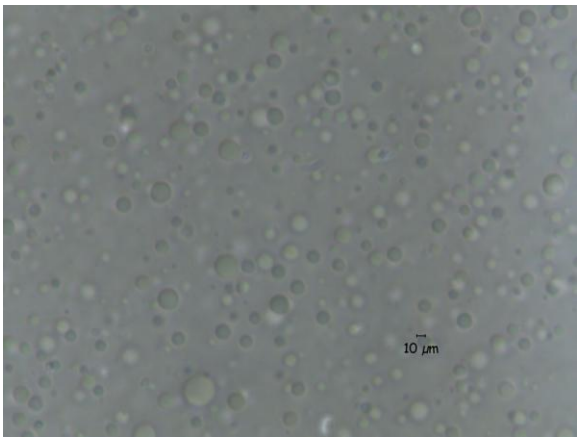


4.2-a

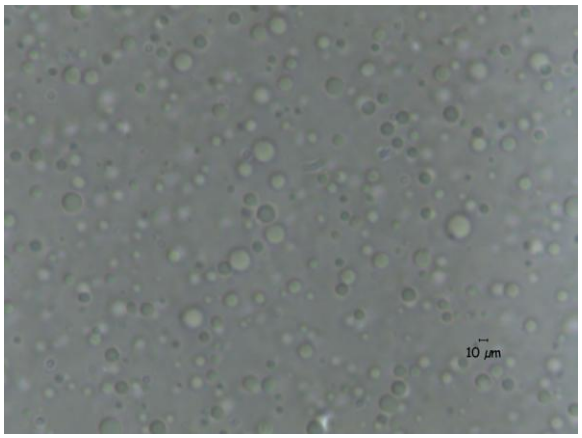


4.2-b

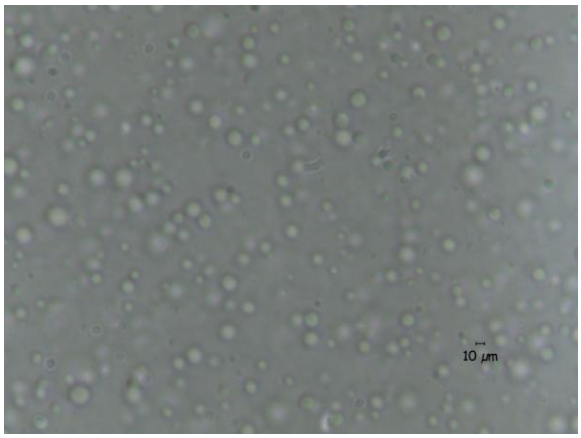
Figure 4.2 Turbiscan back scattering profiles formulated with representative SG and SY @ 25°C (4.2-a: SG2; 4.2-b: SY1). These data are represented as a function of time (hr: min) and of sample height (mm).



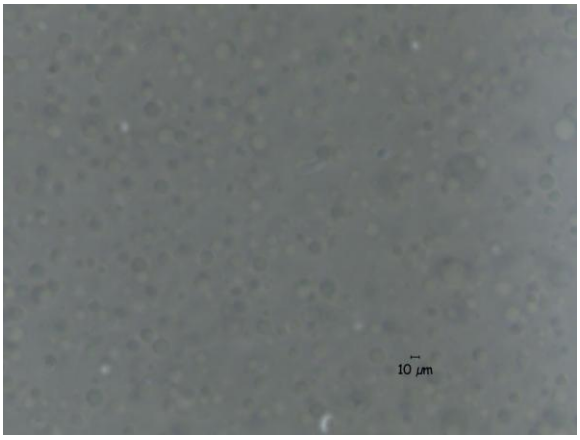
SG1-day 0



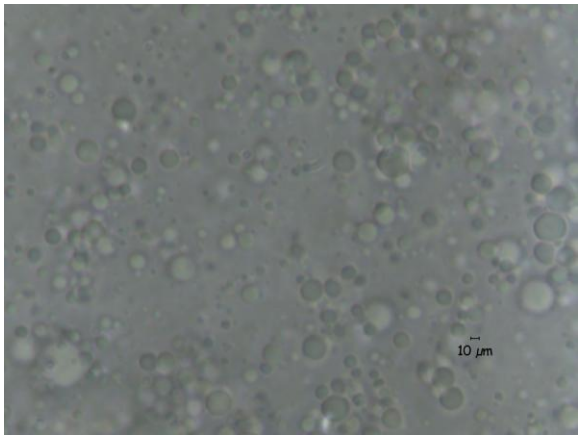
SG2-day 0



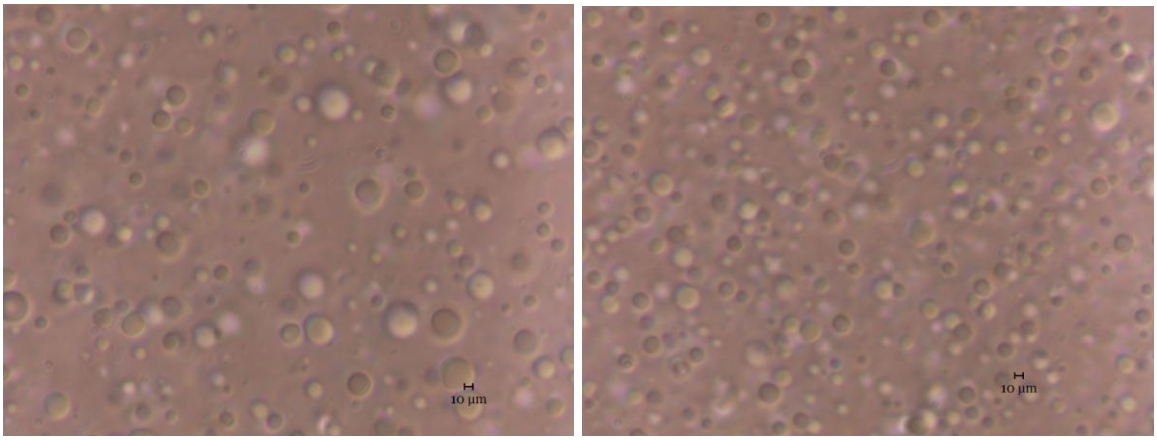
SG3-day 0



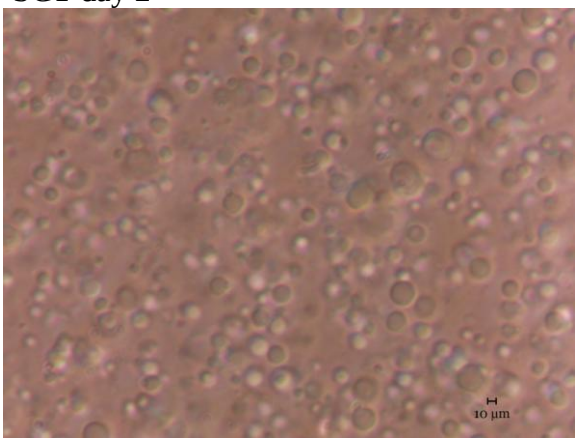
SY1-day 0



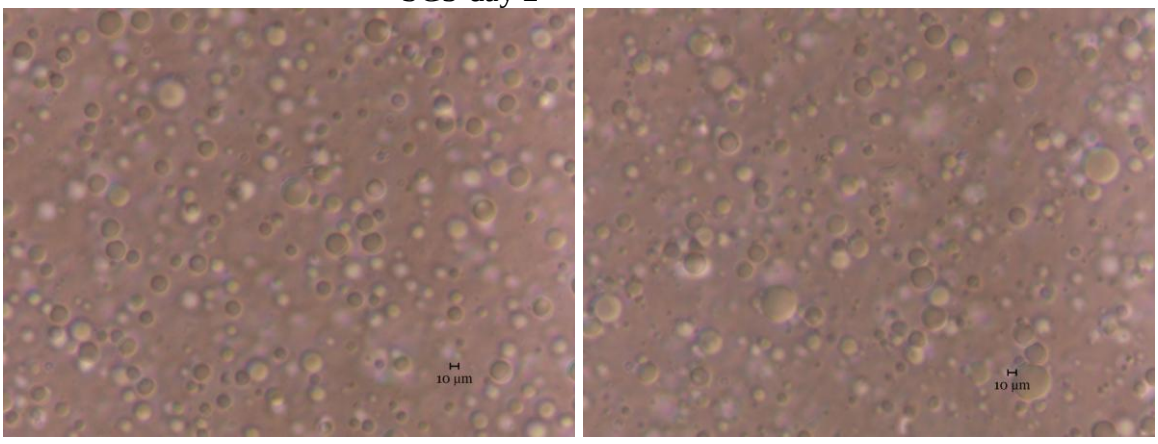
SY2-day 0



SG1-day 2 SG2-day 2



SG3-day 2



SY1-day 2 SY2-day 2

Figure 4.3. Light Microscopy pictures of emulsion particulates formulated with SG1, SG2, SG3, SY1 and SY2 stored at 4°C at day 0 and day 2 (legend in a 10 µm scale)

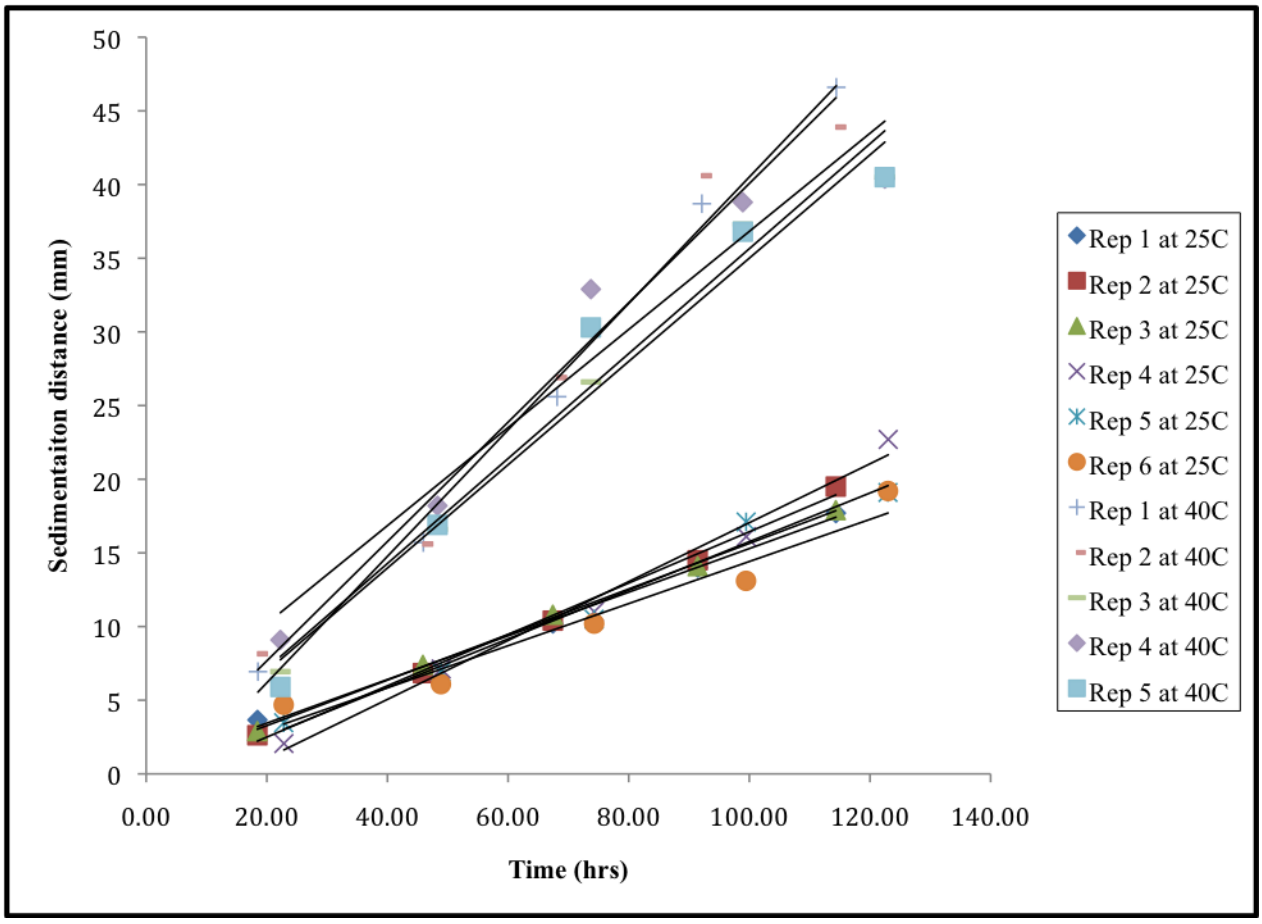


Figure 4.4 Representative of comparison of sedimentation rates (mm/hr) (All replications of SG1as the representative at 40°C and 25°C). These data are represented as a function of time (hrs) and of sedimentation distance (mm at -10% absorbance after subtraction from Day 0).

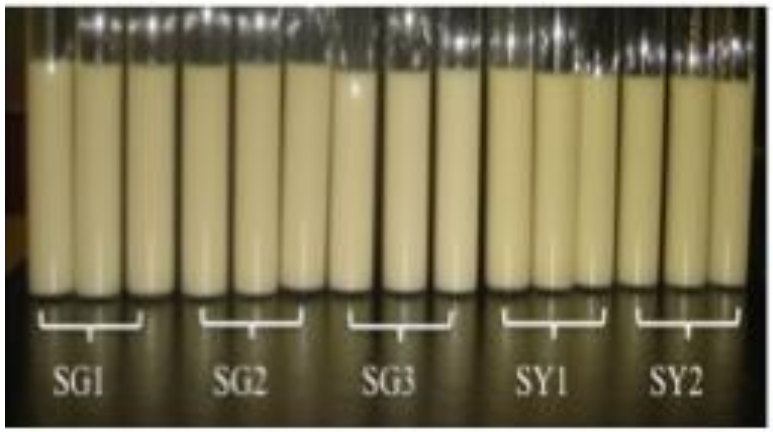
Table 4.1 Slope (m) and correlation coefficient (R^2) of sedimentation rates (mm/hr) of all gum samples stored at 25°C and 40°C [a]

Storage temp.	m & R^2	SG			SY	
		SG1	SG2	SG3	SY1	SY2
25°C	m	0.164^{a}±0.021	0.109^{b}±0.020	0.133^{c}±0.008	0.134^{c}±0.019	0.124^{c}±0.024
	R^2	0.985±0.018	0.957±0.039	0.979±0.006	0.959±0.037	0.987±0.010
40°C	m	0.375^{a}±0.040	0.280^{b}±0.035	0.345^{c}±0.027	0.267^{b}±0.131	0.133^{d}±0.033
	R^2	0.968±0.022	0.941±0.073	0.984±0.016	0.923±0.066	0.942±0.073

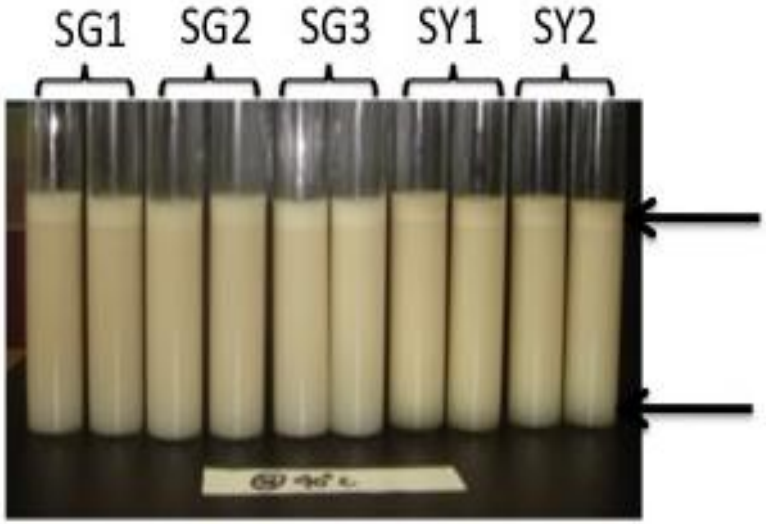
[a] All values are presented as mean ± standard deviation of six measurements from triplicate experiments

[a] Within in a row for SG samples, they showed significantly different m (slope) values ($p<0.05$), in the order as m (SG2)<m (SG3)<m (SG1) and m (at 25°C)<m (at 40°C).

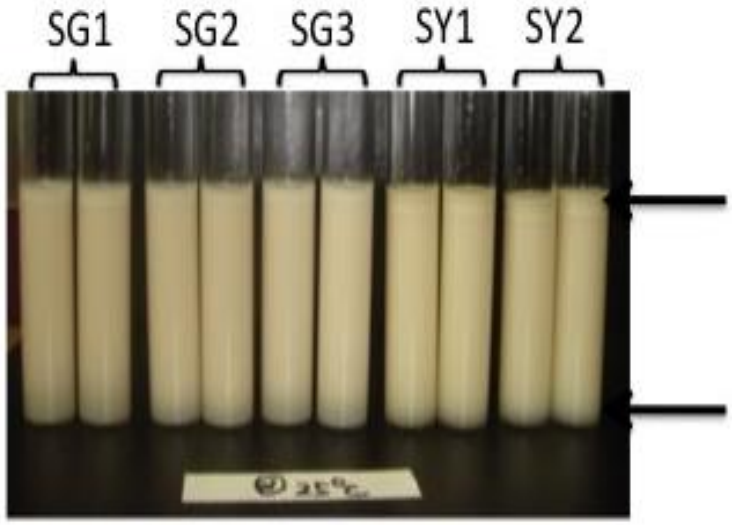
[a] Within in a row for SY samples, they showed no significantly different m values ($p>0.05$) at 25°C but significant different m values ($p<0.05$) at 40°C.



4.5-a



4.5-b



4.5-c

Figure 4.5 Emulsions formulated with SG1, SG2, SG3, SY1 and SY2 (4.5-a: tubes at day 0; 4.5-b: tubes at day 6 stored at 40°C; 4.5-c: tubes at day 6 stored at 25°C

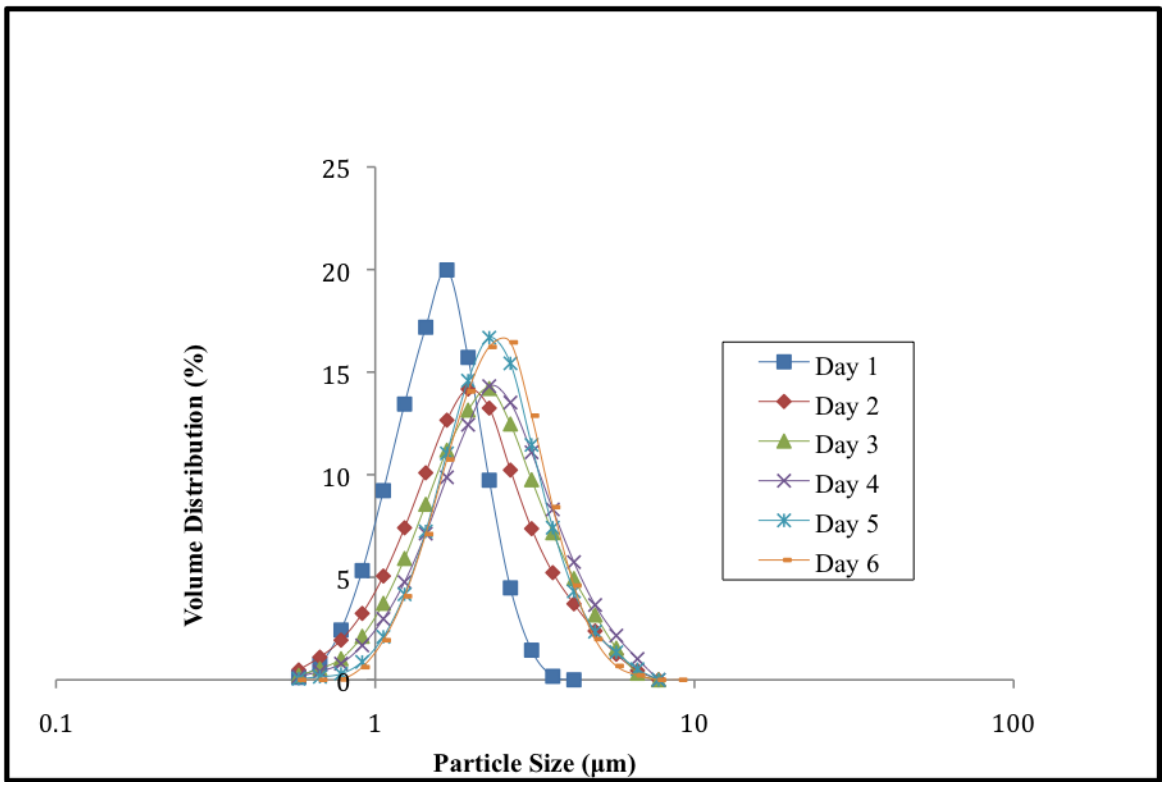


Figure 4.6-a

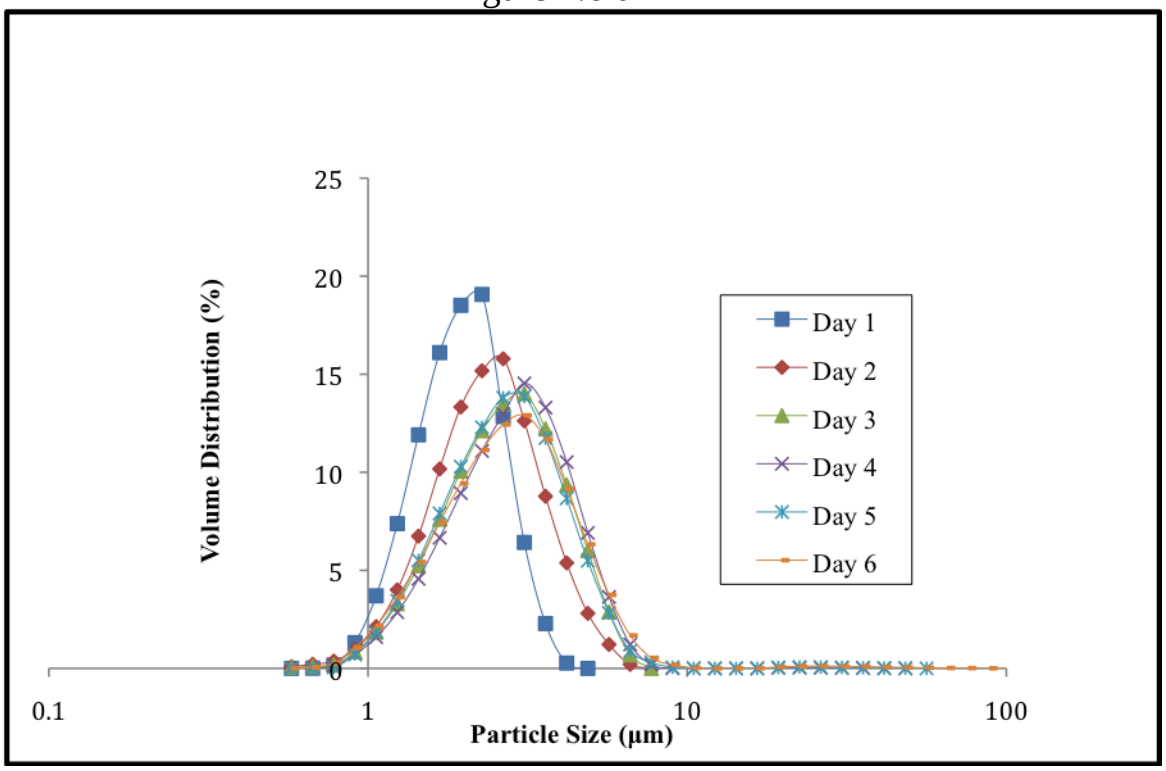


Figure 4.6-b

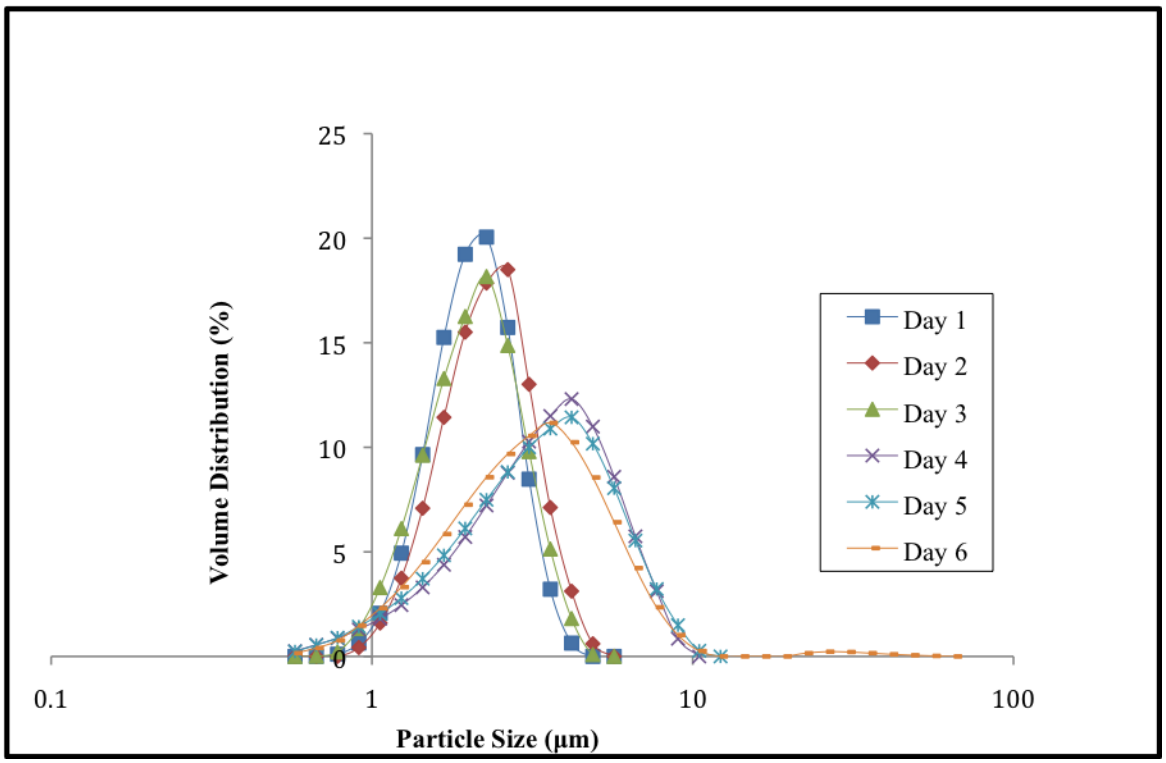


Figure 4.6-c

Figure 4.6. Particle size distribution of emulsions formulated with SG representative (SG1) stored at three different temperatures for 6 days (Figure 4.6-a: stored at 4 °C; 4.6-b: stored at 25 °C; 4.6-c: stored at 40 °C)

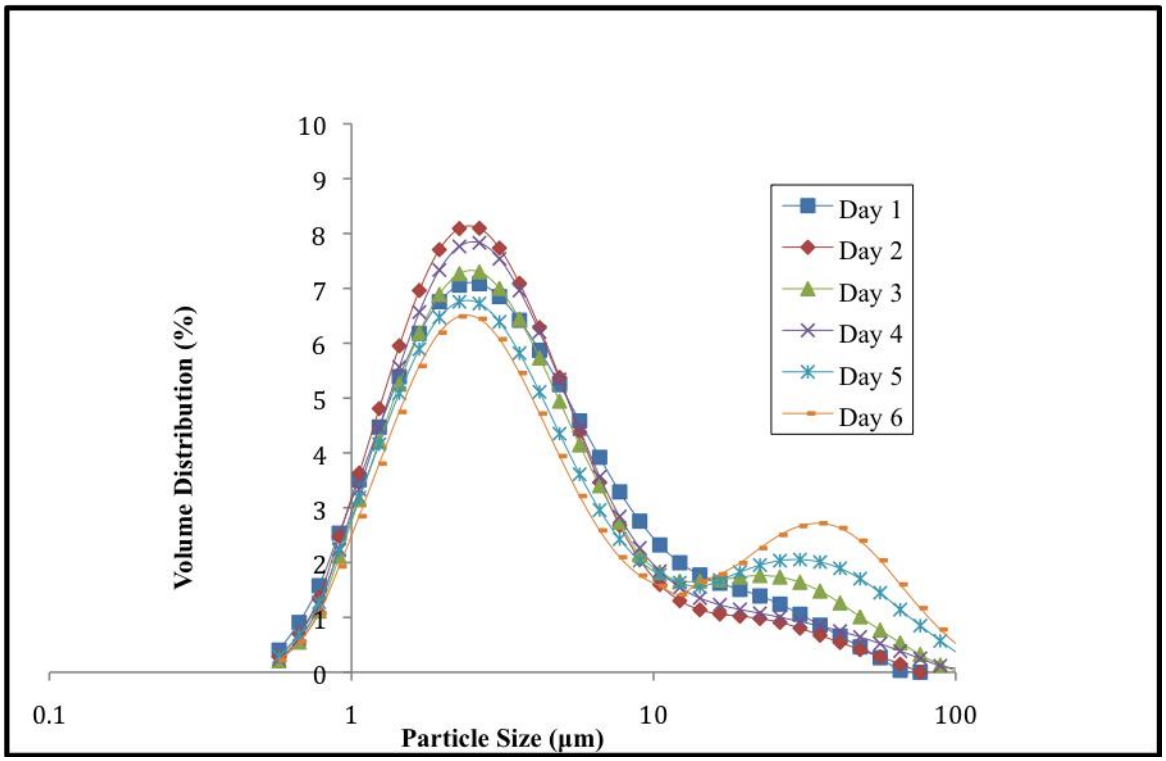


Figure 4.7-a

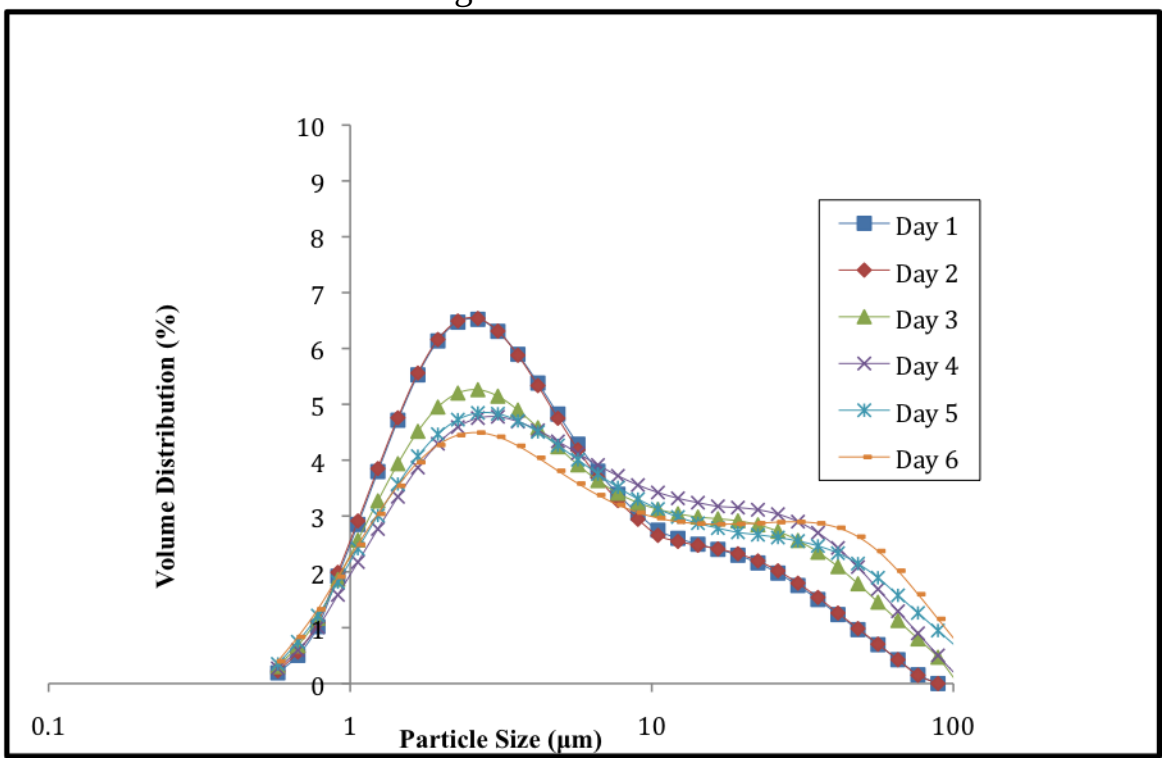


Figure 4.7-b

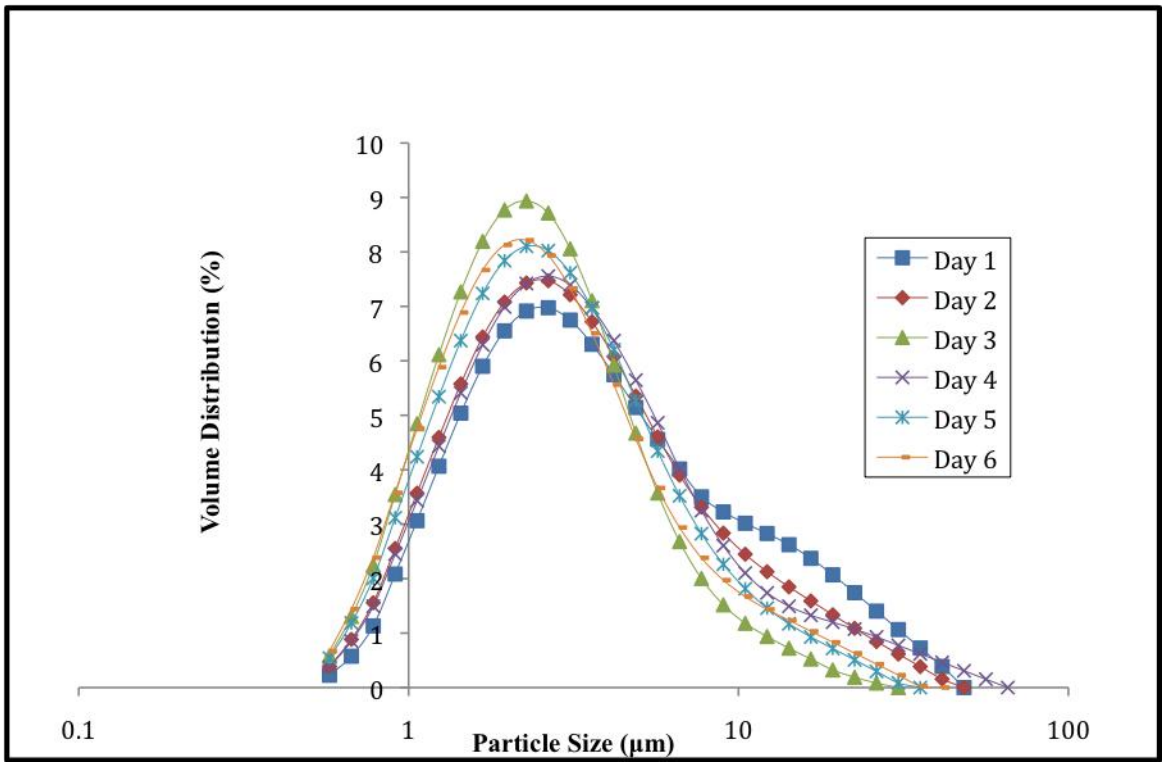


Figure 4.7-c

Figure 4.7. Particle size distribution of emulsions formulated with SY representative (SY1) stored at three different temperatures for 6 days (Figure 4.7-a: stored at 4 °C; 4.7-b: stored at 25 °C; 4.7-c: stored at 40 °C)

Table 4.2 Malvern $D_{4,3}$ and $D_{3,2}$ values for all gum samples stored at 4, 25 and 40°C [a]

Storage temp.	D value	Days	SG			SY	
			SG1	SG2	SG3	SY1	SY2
@4°C	$D_{4,3}$	1	1.50±0.00	1.53±0.01	1.52±0.01	5.56±0.42	6.11±0.02
		2	2.04±0.28	1.85±0.10	1.96±0.18	4.88±0.01	5.73±0.11
		3	2.22±0.28	2.09±0.13	2.13±0.18	7.29±0.33	6.82±0.48
		4	2.37±0.29	2.21±0.09	2.29±0.18	5.91±0.59	12.44±0.04
		5	2.29±0.31	2.16±0.15	2.25±0.20	9.90±0.44	8.11±0.01
		6	2.30±0.19	2.26±0.09	2.17±0.03	11.38±0.01	12.97±0.42
	$D_{3,2}$	1	1.36±0.00	1.40±0.02	1.38±0.01	2.32±0.08	2.39±0.02
		2	1.65±0.08	1.53±0.12	1.61±0.04	2.22±0.01	2.37±0.13
		3	1.83±0.09	1.68±0.08	1.76±0.12	2.49±0.02	2.39±0.03
		4	1.95±0.08	1.80±0.09	1.89±0.07	2.34±0.03	2.76±0.18
		5	1.98±0.11	1.85±0.09	1.94±0.03	2.55±0.01	2.32±0.06
		6	2.03±0.10	1.96±0.02	1.94±0.03	2.77±0.18	2.62±0.09
@25°C	$D_{4,3}$	1	1.87±0.01	1.86±0.01	1.83±0.01	7.65±0.25	5.33±0.01
		2	2.34±0.24	2.26±0.16	2.32±0.20	7.66±0.06	7.82±0.25
		3	2.66±0.18	2.48±0.11	2.52±0.14	10.93±0.20	12.21±0.01
		4	2.82±0.08	2.63±0.06	2.69±0.09	12.16±0.43	11.22±0.34
		5	2.72±0.31	2.50±0.25	2.68±0.04	13.23±0.39	10.26±0.18
		6	2.95±0.57	2.70±0.35	2.60±0.28	14.78±0.47	17.25±2.75
	$D_{3,2}$	1	1.70±0.00	1.70±0.01	1.69±0.01	2.68±0.06	2.26±0.00
		2	2.01±0.07	1.95±0.04	1.99±0.05	2.65±0.03	2.57±0.04
		3	2.23±0.07	2.13±0.03	2.14±0.07	2.99±0.04	2.98±0.04
		4	2.35±0.06	2.24±0.06	2.27±0.05	3.30±0.04	3.10±0.01
		5	2.25±0.17	2.17±0.16	2.23±0.08	3.14±0.16	2.63±0.00
		6	2.27±0.22	2.24±0.14	2.17±0.17	3.18±0.10	3.28±0.34
@40°C	$D_{4,3}$	1	2.13±0.00	2.18±0.01	2.17±0.00	5.65±0.11	5.68±0.03
		2	2.57±0.32	2.52±0.24	2.58±0.26	3.35±0.12	4.23±0.06
		3	2.62±0.47	2.56±0.35	2.53±0.39	2.97±0.23	4.35±0.47
		4	3.06±0.27	2.79±0.34	2.80±0.42	3.75±0.01	3.49±0.46
		5	2.90±0.39	2.66±0.43	2.57±0.54	3.19±0.40	3.94±0.08
		6	2.60±0.62	2.36±0.53	2.31±0.60	3.10±0.53	3.14±0.53
	$D_{3,2}$	1	1.97±0.01	1.98±0.00	1.99±0.00	2.51±0.03	2.49±0.01
		2	2.18±0.15	2.17±0.08	2.17±0.09	2.29±0.09	2.07±0.24
		3	2.15±0.28	2.17±0.22	2.10±0.22	2.21±0.08	1.99±0.33
		4	2.31±0.20	2.19±0.22	2.14±0.28	2.34±0.08	1.87±0.31
		5	2.20±0.24	2.13±0.23	2.01±0.32	2.22±0.16	1.90±0.16
		6	1.98±0.31	1.92±0.35	1.84±0.37	2.06±0.18	1.68±0.17

[a] All values are presented as mean ± standard deviation of six measurement from triplicate experiments

[a] One-way ANOVA showed that for the specific gum sample stored at specific temperature, $D_{4,3}$ in 6 days were significant different ($P<0.05$) , and $D_{3,2}$ in 6 days were significant different ($P<0.05$)

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CHAPTER 5

CONCLUSIONS

There is a need for a better understanding of gum acacia in structural characterization and functionality, especially the gum of commerce, which is processed before being sold. It is clear that variability exists among different gum products due to the origins of gum trees and processing methods.

This research indicated that surface hydrophobicity using a Flouorolog-2 Spectrofluorometer to measure ANS fluorescence relative intensity had a high correlation coefficient with gum concentration for all types of gum samples, with significantly statistical differences. Raman and FT-IR spectroscopic techniques to detect gum products functional groups can be useful tools to predict gum functionality. It also presented that the two species of gum acacia, *Acacia senegal* and *Acacia seyal*, performed differently in destabilization process.

At the beginning of storage period, SG samples were more stable and had less creaming than SY samples. SG and SY had different shifts. SG samples PSD had a shift towards particle sizes. SY samples PSD had a shift toward the volume distribution, and with larger $D_{4,3}$ values. Overall, SG sample was superior to SY, and especially, SG2 performed better than other SG samples.