

Chapter One

Introduction

1-Introduction

1. 1. Natural gums or Hydrocolloids

These materials are exudates from trees or bushes, extracts from plants or seaweeds, flours from seeds or grains, gummy slimes from fermentation processes, and many other natural products. Occurrence of a large number of hydroxyl groups noticeably increases their affinity for binding water molecules rendering them hydrophilic compounds. Further, they produce a dispersion, which is intermediate between a true solution and a suspension, and exhibits the properties of a colloid. Considering these two properties, they are appropriately termed as ‘hydrophilic colloids’ or ‘hydrocolloids’.

Hydrocolloids or gums are a diverse group of long chain polymers characterized by their property of forming viscous dispersions and/or gels when dispersed in water (Jafar Milani and Gisoo Maleki (2012))

1.2. Types of gums

Gums are polysaccharides that originate from natural raw materials such as plants, algae and microorganisms. Virtually free of fat, gums consist primarily of complex carbohydrates derived from plants or from the biosynthesis of end products by microorganisms (e.g., xanthan gum). Natural gums include agar, alginate, carrageenan, guar, tragacanth, locust bean gum, Gellan gum, Agar, Xanthan gum, Gum karaya, etc, among which the most important one is gum arabic (Coppen1995; Dipjyoti Saha and Suvendu Bhattacharya)

1.3. Gum Arabic(GA)

Acacia gum, also known as gum arabic, is a natural, vegetable exudate from *Acacia* trees(primarily in Africa) known since antiquity and used for thousands of years in foods as an additive and ingredient, in the pharmaceutical industry. Gum arabic or gum *Acacia* is a tree gum exudate and has been an important article of commerce since ancient times. It was

used by the Egyptians for embalming mummies and also for paints for hieroglyphic inscriptions. Traditionally the gum has been obtained mainly from the *Acacia senegal* species. The trees grow widely across the Sahelian belt of Africa situated north of the equator up to the Sahara desert and from *Senegal* in the west to Somalia in the east. The gum oozes from the stems and branches of trees (usually at five years of age or more) when subjected to stress conditions such as drought, poor soil

or wounding. Production is stimulated by ‘tapping’, which involves removing sections of the bark with an axe taking care not to damage the tree. The sticky gummy substance dries on the branches to form hard nodules which are picked by hand and are sorted according to color and size. Commercial samples commonly, contain *Acacia* species other than *Acacia senegal* notably, *Acacia Seyal*. In Sudan the gum from *Acacia Senegal* and *Acacia Seyal* are referred to as hashab and talha respectively. The former is a pale to orange-brown solid which breaks with a glassy fracture and the latter is darker, more friable and is, rarely, found in lumps in export consignments. Hashab is, undoubtedly, the premier product but the lower-priced talha has found recent uses which have boosted its

value. It is not possible to identify, precisely, the exact balance between these two products in the market-place since it is continually, changing(Williams and Phillips, 2000).

1.4. Physicochemical properteis of gum arabic

1.4.1. physical properties:-

Gum arabic (GA) is a heterogeneous material having both hydrophilic and hydrophobic affinities. GA physicochemical responses can be and led depending on the balance of hydrophilic and hydrophobic interactions. GA functional properties are closely related to its structure, which determines, for example, solubility, viscosity, degree of interaction with water and oil in an emulsion, microencapsulation ability, among others.

1.4.1.1 Solubility and viscosity

GA has high water solubility and a relatively low viscosity compared with other gums. Some gums cannot dissolve in water in concentrations above 5% due to their high viscosity. In soluble, GA can get dissolved in water in a concentration of 50% w/v, forming a fluid solution with acidic properties (pH = 4.5). The highly branched structure of GA molecules leads to compact relatively small hydrodynamic volume and consequently GA will, only, become a viscous solution at high concentrations. Solutions containing less than 10% of GA have a low viscosity and respond to Newtonian behavior (Williams et. al., 1990). However, steric interactions of the hydrated molecules increase viscosity in those solutions containing more than 30% of GA resulting in an increasingly pseudo plastic behavior. Its high stability in acidic solutions is exploited to emulsify citrus oils. The viscosity of GA solutions can be modified by the addition of acids or bases as these ones change the electrostatic charge on the macromolecule. In very acidic solutions, acid groups are neutralized inducing a more compact conformation of the polymer which leads to a decreased viscosity; while a higher pH (less compact molecule) results in maximum viscosity around pH 5.0-5.5. In very basic solutions, the ionic strength increment reduces the electrostatic repulsion between GA molecules producing a more compact conformation of the biopolymer and thus reducing the viscosity of the solution (Anderson et. al., 1990; Williams et. al., 1990).

1.4.1.2 Emulsifying properties

GA is well recognized as emulsifier used in essential oil and flavor industries. Randall(1998) reported that the arabinogalactan protein(AGP) complex is the main component responsible for GA ability to stabilize emulsions by AGP, amphiphilic protein component association with oil droplets the oriented surface while the hydrophilic carbohydrate fraction is toward the aqueous phase, preventing droplets aggregation by electrostatic repulsion , . However,

only 1-2% of the gum is absorbed into the oil-water interface and participates on emulsification; thus, over 12% of GA content is required to stabilize emulsions with 20% oil (www.intechopen.com; Williams et al., 1990). If there is not enough GA amount to cover all the oil drops, unstable emulsion is formed and flocculation and coalescence occurs.

1.4.1.3. Molecular association

The tendency of polysaccharides to associate in aqueous solution it is well known. These molecular associations can deeply affect their function in a particular application due to their influence on molecular weight, shape and size, which determines how molecules interact with other molecules and water. There are several factors such as hydrogen bonding, hydrophobic association, an association mediated by ions, electrostatic interactions, which depend on the concentration and the presence of protein components that affect the ability to form supramolecular complexes. Al-Assaf (2005), showed that molecular associations in GA can lead to an increase in molecular weight in the solid state by maturation under controlled heat and humidity. The process does not involves change in the basic structural components, while the maturation takes place, the level of association increases giving way to AGP with higher molecular weight and protein content. This process mimics the biological process which produces more AGP throughout the tree growth, and gets maturation to continue during the storage of GA after harvest. Subsequently, Al-Assaf, analyzed the role of protein components in GA to promote molecular association when the gum is subjected to different processing treatments such as maturation, spray drying and irradiation. Results demonstrate the ability of protein components to promote hydrophobic associations that influence the size and proportion of the high molecular weight component AGP. When GA undergoes maturation (solid state heat treatment) there is an increase in the hydrophobic nature of the gum and therefore an increase of its emulsifying properties (Al-Assaf (2005).

Spray drying involves not only the aggregation through hydrophobic associations but also changes in the surface properties of peptide residues increasing GA hydrophilicity compared with the association promoted by the treatment of maturity in the solid state. Ionizing radiation in both aqueous solutions and solid state induces cross-linking between polysaccharide blocks by the formation of –C-C- bonds. It was also reported that, using mild UV-radiation, it is possible to induce GA crosslinking (Kuan(2011)). The process reduced the solution viscosity and improved emulsification properties. This GA modification can be used in food products requiring better reduced viscosity emulsifying properties such as dressings, spreads, and beverages, as well as in other non food products such as lithographic formulations, textiles, and paper manufacturing.

1.4.2. Chemical properties A gums:

The chemical composition of GA may vary slightly depending on its origin, climate, harvest season, tree age and processing conditions, such as spray dying (Al-Assaf, et al., 2005 (a,b); Flindt et al., 2005; Hassan et al., 2005; Siddig et al., 2005). Therefore, there are some differences between the chemical composition of the GA taken from *Acacia Senegal* and *Acacia seyal*. In fact, both gums have the same sugar residues but *Acacia seyal* gum has a lower content of rhamnose and glucuronic acid and a higher content of arabinose and 4-O-methyl glucuronic acid than *Acacia senegal* gum. Instead, *Acacia seyal* gum contains a lower proportion of nitrogen, and specific rotations are also completely different(*Acacia senegal*, -30 degree and *Acacia seyal*, +51 degree). The determination of the latter parameters may clearly spot the difference between the two. Table(1) presents the chemical composition and some properties of both gums reported by Osman and others (Osman et. al., 1993; Menzies & Phillips 1993). Despite having different protein content, amino acid composition is similar in both gums. Recently, Mahendran etal(2008) reported that GA amino acid composition in *Acacia*

senegal, being rich in hydroxyproline, erine, threonine, leucine, glycine, histidine .

Table(1): chemical composition of the main types of Gum Arabic Osman et.al.,1993

No_	Component	Acacia senegal	Acacia seyal
1	%Rhamnose	14	3
2	%Arabinose	29	41
3	%Galactose	36	32
4	%Glucuronic acid	14.5	6.5
5	%Nitrogen	0.365	0.147
6	%Protein	2.41	0.97

1.5.Application of Gum Arabic

Gum Arabic, an has a unique combination of excellent emulsifying properties and low solution viscosity. These properties make gum arabic very useful in several industries , especially, in the food industry where it is used as a flavor encapsulater and stabilizer of citrus oil emulsion concentrates in soft drinks.(Madhav et.al.,(2007)

Gum arabic as a typical and ideal Hydrocolloid has a wide range of functional properties in foods including; thickening, gelling, emulsifying, stabilization, coating and etc (Verboken, Dierckx, and Dewettinck,(2003). Hydrocolloids have a profound impact on food properties when used at levels ranging from a few parts per million for carrageenan in heat-treated dairy products to high levels of acacia gum, starch or gelatin in jelly confectionery. The primary reason behind the ample use of hydrocolloids in foods is their ability to modify the rheology of food systems. This includes two basic properties of food systems that is, flow behavior (viscosity) and mechanical solid property (texture). The modification of texture and/or viscosity of food systems helps modify its sensory properties, therefore hydrocolloids are used

as significant food additives to perform specific purpose. In the food industry, gum Arabic is primarily used in confectionery, bakery, dairy, beverage, and as microencapsulating agent.(McNamee, O'Sullivan, & O'Riordan, 1998; Verbeken, Dierckx, and Dewettinck, (2003)

1.5.1.Confectionery:

The major application of gum Arabic is in the confectionery industry here it is used in variety of products including gums, pastilles, marshmallows and toffees. Gum arabic 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 low calories candie, gum is used to compensate for the loss of texture and mouth feel, 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 and 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 During the preparation, the gum is dissolved in water keeping the temperature as low as possible (~60°C) in order to avoid precipitation of the proteinaceous components which would give rise to a turbid solution. The gum is then added to a pre-boiled sugar/glucose solution (70%) followed by the flavorings and colors. After standing to allow air bubbles to rise, any surface scum is removed and the liquid deposited into starch trays which are placed in a stoving room for 4–6 days. The gums are then taken from the moulds, brushed to remove starch and often glazed with oil or wax. Softer gums or pastilles can be obtained by reducing the stoving time to 2–3 days.

In recent times, because of gum shortages and price fluctuations, considerable efforts have been made to find replacements for gum arabic and nowadays pastilles are prepared using gum arabic at much lower concentrations in combination with other hydrocolloids, notably starch, maltodextrin, gelatin, pectin and agar. In these formulations demixing may occur due to incompatibility between the various hydrocolloids. The extent of demixing will depend on the rate of gel formation induced by the other hydrocolloids present and will consequently dictate the final texture obtained. In marshmallows the gum is used as a foam stabilizer while in toffees it is used to emulsify the fats present. Gum arabic is also used to form a glaze on coated nuts and similar products. (Verbeken, Dierckx, and Dewettinck, 2003)

1.5.2.Beverages

Gum Arabic is stable in acid conditions and is widely used as an emulsifier in the production of concentrated citrus and cola flavor oils for application in soft drinks (Verbeken, Dierckx, & Dewettinck,(2003) Eng and Mackenzie, 1984; Phillips, 1998). The gum inhibits flocculation and coalescence of the oil droplets over several months and furthermore the emulsions remain stable for up to a year when diluted up to ~500 times with sweetened carbonated water prior to bottling. In the preparation of the emulsion a weighting agent is, normally, added to the oil in order to increase the density to match that of the final beverage and thus inhibit creaming. Typical weighting agents that are used, subject to legislation in various parts of the world, are glycerol ester of wood, gum damar and sucrose acetate isobutyrate, which is not normally used by itself but, usually, in conjunction with rosin or gum damar. The emulsion is prepared by adding the oil to the gum arabic solution under high speed mixing followed by homogenization yielding a droplet size of ~1 micron. A typical formulation might contain 20% gum Arabic, 10% flavor oil and 5% weighting agent while the final beverage might contain 0.1–0.2% concentrated emulsion, 10% sugar and 0.2% citric acid/coloring.

1.5.3. Flavor encapsulation

Microencapsulation is commonly used to transform food flavors from volatile liquids to powder particles that can be, readily, incorporated into dry food products such as soups and dessert mixes. The process also renders the flavor stable to oxidation. Encapsulation involves spray-drying an emulsion of the flavor oil which is produced using gum Arabic as emulsifier. Nowadays malt dextrin is commonly mixed with the gum in order to reduce costs. (Williams and Phillips,2000)

1.6. Emulsion and Emulsification

An emulsion is a heterogeneous system consisting of at least one immiscible liquid dispersed in another in the form of droplets(Friberg et. al., 1969). Emulsions are classified based on the nature of the emulsifier(e.g. phospholipids ,proteins, and polysaccharides) or the structure of the system. Accordingly, There are several type of emulsion systems, the most important of which, that find its way to wide and important application is Oil-in-Water emulsions which consist of small oil droplets dispersed within an aqueous environment.

.1.6.2Classification of Emulsion

Emulsions play an important role in the food industry in terms of product appearance, texture, flavor and stability. By definition, an emulsion is comprised of a mixture of two immiscible liquids with one liquid dispersed as small droplets within the other, formed after applying mechanical agitation (i.e., homogenization) in the presence of an emulsifier. Depending on the nature of the dispersed liquid, emulsions can be classified as water-in-oil (W/O) or oil-in-water (O/W). An emulsifier is a small (i.e., lecithin) or large (i.e., protein) amphiphilic molecule that migrates to the oil-water

interface to lower interfacial tension .

Emulsions may be classified according to the nature of the emulsifier or the structure of the system. A number of different terms are commonly used to describe different types of emulsions and it is important to classify these terms (McClements, 2010; Solan et al, 2005; Maron et al, 2006; Tadros et al., 2004), among which is the droplet size. The range of droplets size for each type of emulsion is quite arbitrary, differentiating them on the basis of size and stability. Macro emulsions are the most common form of emulsions used in food industries than nano and micro emulsions.(Zhang et. al., 2008). Emulsions may also be classified according to their physical and thermodynamic properties. A conventional emulsion, also known as a macroemulsion, typically has a mean droplet diameter between 100nm and 100µm. Macroemulsions are the most common form of emulsions used in food industry and are found in a variety of products, including milk, beverages, mayonnaines, dips, sauces and desserts. Macroemulsions are prone to physical instability (e.g. gravitational separation, foculation and coalescence). Especially when exposed to environmental stresses(Peter, 2009; Djord Jevic et. al., 2007). Nanoemulsion are dispersions of nano scale droplets with a mean droplet diameter between 20-100nm. (Landfester et al, 2000). In contrast to nanoemulsions, miniemulsion have droplets in the range from 100nm to 1µm. Because miniemulsions and macroemulsions are thermodynamically, unstable. Microemulsion is a thermodynamically, stable system and forms, spontaneously, with droplet size between 5 to 50nm (McClements, 2010). Nanoemulsions are the same as microemulsions since they both typically, contain oil, water and surfactant and also have similar mean droplet diameter

.However, the two systems are very different (Zhang et al, 2008; Garti et al, 2004), where the main difference between microemulsion and miniscale

emulsion is not composition but rather thermodynamic behavior (Whitesides, 2002).

1.6.3. Emulsion Stability

Emulsions play an important role in the food industry in terms of product appearance, texture, flavor and stability. Emulsions are chemical mixtures of liquids that are immiscible under ordinary conditions, and in the absence of emulsifying and stabilizing agents may separated into two layers on standing, heating, and freezing, by agitation or the addition of other chemicals (Encyclopedia of chemical technology (1966)).The emulsifying agents act as surface-active agents, which when added to an emulsion it would increase its stability by interfacial action.

Small emulsifiers diffuse quickly to the interface, promoting emulsion formation and short-term stability. Large emulsifiers migrate at a slower pace, then re-align (undergoing a conformational change) at the interface to form a viscoelastic film around the dispersed droplets. Depending on the nature of the emulsifier present and solvent conditions, coalescence may be inhibited by steric hindrance and electrostatic repulsion between neighboring droplets.

Emulsion stability is dependent on several parameters, including: (i) type of emulsifier present; (ii) droplet size (smaller droplets are more stable); (iii) continuous phase viscosity (a viscous continuous phase reduces the rate of creaming); and (iv) the volume ratio of dispersed versus continuous phase. In general, proteins aid in emulsion stability by interacting with the oil-water interface and by increasing the continuous phase viscosity, whereas polysaccharide functions primarily by the latter mechanism (Dickinson, E. , 2003). For admixtures of proteins and polysaccharides, emulsion stability or instability may be favored depending on the biopolymer present, solvent conditions and the method of emulsification. Biopolymers can be mixed prior

to emulsification (i.e., biopolymer complexes interacts with the oil-water interface) or one biopolymer is added (i.e., polysaccharide) to an already protein stabilized emulsion (i.e., biopolymers interact through a layer-by-layer assembly approach, with only one biopolymer interacting with the interface) (Jourdain, et. al. 2008). In the latter case, emulsion stability becomes concentration dependent. At a dilute polysaccharide concentration, bridging flocculation between protein-coated droplets may occur and lead to emulsion destabilization. In contrast, emulsion stability is enhanced when the polysaccharide concentration is sufficiently high to completely cover protein-coated droplets; or if in excess, a weak gel-like network may form that immobilizes the droplets (Dickinson, E. , 2003).

In emulsions, the lipid droplets are normally coated by a thin layer of emulsifier molecules to prevent them from aggregating. In the food industry, a variety of different kinds of surface-active molecules are used as emulsifiers, including small molecule surfactants, phospholipids, proteins, and polysaccharides. (Jafar and Gisoo , 2012) Physical instability results in an alteration in the spatial distribution or structural organization of the molecules. Creaming, flocculation, coalescence, partial coalescence, phase inversion, and Ostwald ripening are examples of physical instability. The development of an effective strategy to prevent undesirable changes in the properties of a particular food emulsion depends on the dominant physicochemical mechanism(s) responsible for the changes. In practice, two or more of these mechanisms may operate in concert. It is therefore important for food scientists to identify the relative importance of each mechanism, the relationship between them, and the factors that influence them, so that effective means of controlling the stability and physicochemical properties of emulsions can be established.

Gum arabic is used to stabilize flavor oil emulsions in the dried food mixes (such as soup, cakes, ...etc) and in the soft drinks industry.

Gum arabic produces highly stable emulsions making it very useful in the preparation of oil in water food flavor emulsions particularly for citrus oils (Randall, et.al., 1998; Jaynes, 1985). Some believe that gums are not true emulsifiers. That is, they do not act by means of hydrophilic – lyophobic chemical functionality, they perform as emulsion stabilizers or protectors. Their function is essentially to increase the viscosity of the aqueous phase by thickening it so that it approximates or slightly exceeds that of the oil. In this way the tendency of the dispersed phase to slip or coalesce is minimized, and the emulsion is, so to speak, stabilized (Elgaili., et.al., 1988). Such stabilization is a protective effect based on thickening properties of the gums. In common with most emulsifiers, the AGP complex has a hydrophobic region (protein) and hydrophilic region (carbohydrate). During the formation of oil in water emulsions the protein portion (arabinogalactan) protrudes into the oil phase. The bulk of gum arabic in the form of free AG can improve stability by increasing viscosity of the water (Islam et. al., 1997).

1.7. Functionalities of Gums

In the world of food product development, the term “functional ingredients” is not new. Food product developers rely heavily on these ingredients to enhance, thicken, stabilize and give products a certain desired texture, appearance or nutrition profile, there are a number of functional ingredients, gums and starches, also referred to as “hydrocolloids,” are just some of these types of ingredients. As mentioned before, the main components of gums are protein and polysaccharides, each of these play important roles in emulsions.

1.7.1. Functions of Proteins

Gum Arabic has been separated into five molecular mass fractions using gel permeation chromatography (Randall et. al. 1989). Each fraction was shown to be present in varying proportions. A high proportion of the proteinaceous material (60%) is associated with one high molecular mass

component, which constitutes <10% of the total gum. This fraction can be degraded enzymatically to give products with similar molecular masses to the other fractions. Although gum arabic solutions of >12% (w/w) were required to give stable 20% (w/w) orange oil emulsions of small droplet size, it was demonstrated that only 1–2% of the gum actually adsorbed at the oil-water interface, gum Arabic increases density of oil droplet through steric stabilization and reduces the surface tension between water and oil, and hence increases the stability of emulsions. Further investigation revealed that it was the high molecular mass, protein-rich fraction which predominantly adsorbed and hence is responsible for the gum's emulsifying ability. Stable emulsions could not be produced using enzyme-degraded gum Arabic (Damodaran, 2005). Formation and stabilization of oil-in-water emulsion requires the presence of a surfactant that can effectively reduce the interfacial tension between the oil and aqueous phases such as lecithins, monoacylglycerol or macromolecules such as proteins (Randall et. al. 1989). However, proteins are generally less surface active than small surfactants due to their complex structural properties. Because of conformational constraints to properly orient the hydrophilic and hydrophobic groups at the interface and improper packing at the interface, proteins are unable to greatly reduce the interfacial tension. Although proteins are not highly effective in reducing the interfacial tension, protein-stabilized emulsions are generally more stable than those stabilized by small surfactants (Damodaran, 2005). Proteins form a gel-like film around oil droplets via non-covalent interactions (Dickinson, 2001). Conformational changes in the protein at the interface promote polymerization via the sulfhydryl-disulfide interchange reaction (Damodaran and Anand, 1997). These interactions, apart from making the protein irreversibly adsorbed to the interface, provide a highly viscoelastic film that resists coalescence (Dickinson, 2001). Proteins are good as emulsifying agents and stabilizers when solubility is good and the aqueous environment is suitable for steric and

electrostatic stabilization (Dickinson and Stainsby, 1982). However, the emulsifying activity may be lost close to the isoelectric point of a protein due to the precipitation or aggregation of protein (Damodaran, 1996). At this point protein has balanced positive and negative charges and hence the solubility of proteins is minimal. The emulsifying properties of proteins are also lost at high salt concentration due to charge shielding effects (Damodaran, 1996). At low concentrations (< 0.2 M), salts increase the water binding capacity of proteins. This is because hydrated salt ions bind (weakly) to charged groups on proteins. At this low concentration, binding of ions to proteins does not affect the hydration shell of the charged groups on the protein, and the increase in water binding essentially comes from water associated with the bound ions. However, at high salt concentrations, much of the existing water is bound to salt ions, and results in dehydration of the protein. (Damodaran, 2005)

1.7.2.Function of polysaccharides:

Polysaccharides are important ingredients in manifold products. In particular, polysaccharides are found in the ingredients of a wide range of food emulsions, such as mayonnaise and ice-cream. The relevant functionalities originate from several molecular properties of the polysaccharides and their interaction with emulsion droplets and other components in the food systems. Whereas proteins are present primarily as emulsion forming and stabilizing agents, soluble polysaccharides primarily function as thickening and water-holding agents. Polysaccharides can be used in their natural form, but in many cases the functionality is adapted by chemical modifications, for example, to improve the solubility and water binding capacity. A wide range of properties are found among the whole group of polysaccharides, varying from insoluble forms (cellulose) to high swelling power and solubility(starch, guar gum), low viscosity (gum arabic) to high viscosity (guar gum), and no gelling(dextran) to gelling (agar). Gel

formation is often thermo-reversible and the gel may melt on heating (alginates, pectin) or set on heating (some cellulose derivatives).

In the practical application of polysaccharides, one is often confronted with instabilities of the system, such as serum separation and complex-coacervation or incompatibility between the polysaccharide and other biopolymers in the system, such as proteins. In other cases, the interaction with other components can be used to advantage, for example, in the case of mixed gelation behavior, complex formation between polysaccharides and proteins, and the formation of covalent conjugates (Dickinson, 1992).

1.7.3. Polysaccharides as Emulsions Thickeners

One of the main functions of polysaccharides in emulsions is to thicken the continuous liquid phase. The intended effect is, usually, to impart a desired texture (increased viscosity or stiffness) to the system and to reduce buoyancy-driven creaming or sedimentation of the emulsion droplets and other particles in the system. Because of their, highly, swollen molecular structure in solutions, most polysaccharides are very effective in providing high viscosity at low concentrations.

Water soluble polysaccharides are, often, termed hydrocolloids or gums. They enhance viscosity and/or form gels in aqueous systems. Gum Arabic, locust bean gum, tragacanth, pectins and other, naturally, occurring molecules have been used for many years, in part because of their surface activity. They are used in foods as stabilizers, thickening and gelling agents, crystallization inhibitors, and encapsulating agents. These polysaccharide gums occur in nature as storage materials, cell wall components, exudates, and extracellular substances from plants or microorganisms (Dickinson and Galazka, 1986 ; Mitchell and D.A.)

1.8.Emulsification and Emulsifying properties of Gum Arabic

The emulsification properties of gum Arabic are attributed to the presence of a little protein fraction in its composition, GA(notably gum from

A. senegal) is widely used as an emulsifier to stabilize flavor oil emulsions in the soft drinks industry. Anderson (Anderson,(1986) suggested that the superior emulsifying power of gum arabic may be related to the significant proportions (<10 mol%) of terminal groups, which possess hydrophobic centers. However, in explaining emulsification by gums the critical role assumed by the protein component is now recognized.

Randall and coworkers(Randall et al, 1987) showed that >12% gum Arabic solutions were required to stabilize 20% orange oil emulsions and that it was the high molecular mass protein-rich(Williams et al.,(1990) fraction that preferentially adsorbed at the oil–water interface. They postulated that the more hydrophobic polypeptide chain adsorbed at the surface of the oil droplets with the hydrophilic carbohydrate blocks attached to the chain protruding out into the aqueous phase providing an electrostatic barrier inhibiting droplet aggregation (Dickinson et al,(1989) compared the emulsification properties of a number of Acacia gums with varying protein contents. They showed that the limiting interfacial tension was found to be function of the protein content but that there was no correlation between protein content and emulsion stability. The interfacial properties were found to be very dependent on the nature of the oil phase and studies showed that the surface viscosity was reduced only very slowly over many hours on dilution of the bulk phase.

1.9. Factors Affecting Emulsifying Power of Gum Arabic

1.9.1. Molecular basis of emulsifying power of gum Arabic

In recent years, several investigations have been conducted in order to reveal the molecular structure of GA and relate it to its exceptional emulsifying and rheological properties. The chemical composition of GA is complex and consists of a group of macromolecules characterized by a high proportion of carbohydrates (97%), which are predominantly composed of D-galactose and L-arabinose units and a low proportion of proteins (<3%)(

Islam et al., 1997). The volatile matter (Table) determines the nature and degree of polymerization of the compositions contained in sugar (arabinose, galactose and rhamnose) which exhibits strong binding properties to act as emulsifiers and stabilizers in the manufacture of cough syrups in the pharmaceutical industry (Phillips and Williams, 2001.).

Table(2): international specification of Gum Arabic FAO,1990

No_	Property	
1	Moisture (%)	13 - 15
2	Ash content (%)	2 - 4
3	Internal energy (%)	30 - 39
4	Volatile Matter (%)	51 - 65
5	Optical rotation (degrees)	(-26) - (-34)
6	Nitrogen content (%)	0.26 - 0.39

1.9.2. Effect of Chemical Additives

Among proteins, sodium caseinate (Álvarez Cerimedo et. al., (2010) is widely used as an ingredient in the food industry because its functional properties include emulsification, water-binding, fat-binding, thickening, and gelation. It contains a soluble mixture of surface-active caseins. The caseins adsorb rapidly at the oil–water interface during emulsification and provide long-term stability to oil-in-water emulsions due to a combination of electrostatic and steric stabilization. In milk protein-stabilized emulsions, the presence of ethanol can confer increased stability by reducing the interfacial tension between oil and aqueous phases and so producing a lower average droplet size during emulsification. (Applications of Confocal Laser Microscopy) Motivated by separate observations of the sensitivity of emulsion flocculation to Ca^{2+} content and ethanol concentration, Radford et al. (Radford et. al., (2004) investigated the combined roles of ionic calcium

content and ethanol concentration as variables controlling depletion induced flocculation in caseinate emulsion systems.

On the addition of moderate concentration of ethanol (15 wt%), the dense flocculated protein network appeared to be, completely, broken down. At that point the microstructure was made up of discrete flocculated droplets separated by relatively large distances. This difference in microstructure with 15 wt.% alcohol addition, suggested that alcohol enhance stability at this concentration. A single narrow stable (no creaming) region was identified, indicating limited cooperation between calcium ions and ethanol.

1.9.3. Effect of D-Limonene Essential

Essential oils are natural liquid products obtained from plants by hydro or steam distillation. Essential oils components are volatile substances, sensitive to oxygen, light, moisture and heat (Adamiec and Kalemba, 2006 ; Soottitantawat et. al., 2003). Stability of essential oils can be increased by using microencapsulation, which consists of the entrapment or coating of those substances within another material or system. Microencapsulation is of great importance in the flavoring and food industries , since in this technique, flavors in the liquid form are entrapped in a carrier matrix in order to obtain a dry flavor powder, which is easy to handle. The advantages of this technology are not only in providing protection against degradative reactions and prevention of flavor loss, but also promoting the flavor controlled release during food processing and storage(Fernandes et. al., 2008).

Mahdi Jafari and coworkers (2012) in their comparative study to determine the stability of d-limonene emulsions using a novel hydrocolloid (Angum gum) compared with Arabic gum, gum dispersions with maltodextrin were prepared in water (in 1–5% concentrations) and emulsified with 5% and 10% d-limonene using high pressure homogenization. Statistical analysis revealed a significant influence of gum type and gum concentration on emulsion stability.

1.10. Droplet size and Emulsion Stability, Methods of Determination

Droplet size is one of the most important characterization parameters for emulsion system, and parameters relating to droplet size are consequently reported in all studies on these systems. Droplet size determinations are, predominantly, performed to confirm that the desired colloidal size range has been obtained during preparation and that it is retained upon storage or further processing (e.g. during freeze drying or sterilization). Moreover, droplet size significantly affect the material properties of emulsions. droplet sizing results are thus crucial parameters in the development and optimization of preparation processes as well as in the evaluation of emulsion stability. Droplet sizing, however, has also been employed for other purposes: for example, to evaluate the size dependence of the emulsion properties (Bunjes et al., 2000) or to obtain additional information on the droplet shape (Westesen et. al., 2001 ; Jores, 2004).

Emulsions have been studied by numerous techniques, such as particle sizing, microscopy, rheology, among others, to characterize their physical properties. Most of these techniques involve some form of dilution. This dilution disrupts some structures that contribute to destabilization. The ability to study the stability of food emulsions in their undiluted forms may reveal a remarkable difference between their stability. Thetis the case of CLSM and another new techniques such as Turbiscan. The Turbiscan method, allows scan the turbidity profile of an emulsion along the height of a glass tube filled with the emulsion, following the fate of the turbidity profile over time. The analysis of the turbidity profiles leads to quantitative data on the stability of the studied emulsions and allows making objective comparisons between different emulsions. Turbiscan measurements together with dynamic light scattering measurements are two techniques that allow quantifying the microstructures described by CLSM. Alvarez Cerimedo et al. (Alvarez Cerimedo et al.(, 2010) studied emulsions stabilized by sodium caseinate. In

that article the effect of trehalose on emulsion stability was followed by Turbiscan, the microstructure of emulsions was described by CLSM using Nile red as fluorechrome, and the particle size distribution of emulsion droplets was studied by dynamic light scattering. The fat phase appears in red in CLSM images. It was reported that polysaccharides which are not, particularly, surface-active such as xanthan gum and which are, usually, added to the aqueous phase of emulsions as thickening agents to retard instability mechanisms did not affect the size of the emulsion droplets. On the contrary, the fact that particle size diminished on sugar addition does not allow disregarding interactions. In some systems droplet size can be smaller if polysaccharides are present with the protein during homogenization, so the rate of creaming can be reduced as long as there is no bridging flocculation. Interactions between polysaccharides and proteins are based on hydrogen bonding, and dipole-dipole associations, in which the presence of OH-groups plays a predominant role Anderson et. al.,(1990). Beside, in the Biochemistry field, strong interactions between peptides and short oligosaccharides have been recently reported as playing an important role in protein recognition. All the above indicates that sugar-protein interactions are very common.

In order to slow down emulsions destabilization, thickening agents such as polysaccharides and hydrocolloids are frequently used. Alvarez Cerimedo et al.,(2010) it was shown that the effect of trehalose was further than the ability to form viscous solutions since it diminished average particle size values for the same processing conditions. The interactions between protein and sugar also played an important role in stabilization although was not enough to suppress the depletion effect that led to instability of the emulsions formulated with sodium caseinate concentrations from 2 to 4 wt.%.

As mentioned, oil-in-water emulsions are widely used in the food industry to encapsulate lipophilic functional components, such as vitamins, colors, flavors, nutraceuticals, and antimicrobials. They are also used to

provide desirable optical, rheological and sensory characteristics to many types of food products, including beverages, sauces, dips, dressings and deserts.

1.11. Objectives of the research

The main objectives of this research is to compare the emulsifying and stabilizing power of some *Acacia* gums with D_lemonine flavor oil .

Literature Review

Gum Arabic is defined as the dried gummy exudates from the bark of the stems and branches of certain varieties of the *Acacia* tree, such as *A. Senegal*, *A.Sayal*, *A.Tortilis* and other related African species of *Acacia* (Tan, 1990).

Gum Arabic is colorless, tasteless and odorless, candy, ice cream and sweet syrups. McClements, (2005).

Gum Arabic (GA) is a natural food additive. The most important applications of gum Arabic have been as an emulsifier in the food and pharmaceutical industries. Gum Arabic is considered to be the best gum use in dilute oil-in-water emulsion systems (Garti, N., 1999). One important example of which is the use of citrus oils as flavoring agents in soft drinks where the oils are converted into a water-dispersible emulsion (Verbeke et al., 2003)

Chemically, (GA) is a complex mixture of macromolecules of different size and composition (mainly carbohydrates and proteins). The gum consists of three distinct fractions: a high molecular mass arabinogalactan-protein complex (AGP) containing most of the total protein, a glycoprotein (GP) containing the rest of the protein, and a lower molecular mass fraction, an arabinogalactan polysaccharide, which is protein-deficient (AG), the gum has a variable protein content depending on the species (Anderson, 1986). The (AGP) fraction is mostly responsible for the emulsifying properties of the gum as a whole (Randall, et al., 1988). Although the gum as a whole was shown to contain < 2% protein, it was found that most of this was present within one high molecular mass component which constituted <10% of the total. Considering emulsification it was found that only 1-2% of the gum Arabic adsorbed at the oil-water interface and that the portion that predominantly adsorbed was the high molecular mass, protein-rich fraction.

Protein and oil are the most important ingredients in food emulsions. Oil dispersions in the form of small spherical droplets are stabilized in the

aqueous phase by protein in an oil-in-water (O/W) emulsion. The surface-active protein is adsorbed at the interface between oil and the aqueous phase to lower surface tension and prevent oil droplets from coming close enough together to aggregate (Dickinson, and Golding, 1997; Paraskevopoulos, et.al., 2007). Studies by several groups (Randall, et.al., 1988); Mertens and Huyghebaert, 1988) indicate that the emulsifying property of gum and emulsion stability (Garti, 1999), is due to its protein-rich component, particularly the heterogeneous arabinogalactan protein, which is the major component of gum Arabic (Akiyama, et.al. 1984; Osman, et.al. 1993; Osman, et.al., 1995)

The hydrophilic carbohydrate blocks are linked to the protein chain that strongly adsorbs at the oil-water (O/W) interface promoting emulsion stability (Williams et al., 1990), the exposed amino acids of the polypeptide chain, facilitate adsorption onto hydrophobic substrate, and this explains the ability of gum to act as the best emulsifier/stabilizer for oil-in-water emulsions. Today, the properties and features of (GA) have been widely explored and developed, and it is being used to thicken, emulsify and stabilize emulsions in a wide range of industrial sectors such as textiles, ceramics, lithography, cosmetics and pharmaceuticals, encapsulation, food, etc. Regarding food industry, it is used as a stabilizer, thickener and/or an emulsifying agent. Emulsions are the bases of a wide variety of natural and manufactured materials including foods, pharmaceuticals, biological fluids, agrochemicals, petrochemicals, cosmetics, and explosives (Becher, 1985; Becher, 1988.; McClements, 1999).

Many food products in the markets are in the emulsion state such as cheese, milk, salad dressings, sauces, beverages, coconut milk, soft drink, syrup, gummy candies and creams (Gonzalez 1991; McClements, 1999; Verbeken et al., 2003)

In this paper, the emulsifying properties of four *Acacia* gums, with approximately similar protein contents of c. 2%, were investigated. *Acacia* gums have a functional ability to act as emulsifier that stabilizes oil-in-water emulsion Yokoyama et al., 1988.; Randall et al.,(1988).

An emulsion is a dispersed system that consists of two immiscible liquids (usually oil and water), with one of the liquids dispersed as small droplets in the other called continuous phase(McClements,(1999). The emulsions are thermodynamically unstable systems and have tendency to break down over time (Dickinson, 1992; Friberg and Larsson, 1997; McClements, 1999).

From a physiochemical point of view, emulsions are thermodynamically unstable systems. Over a period of time, an emulsion can rapidly or slowly separate into two immiscible phases. The breakdown of an emulsion may attributed to different physicochemical mechanisms such as gravitational separation, coalescence, flocculation, Ostwald ripening and phase inversion (Tcholakova et.al. 2006; Friberg and Larsson, 1997; McClements, 2000), therefore, the production of high quality food emulsions that can remain kinetically stable for a certain period of time is necessary. For a period of time to increase shelf-life is one of the main challenges of food product formulation, this can be achieved through the addition of emulsifiers and stabilizer, emulsifiers are surface-active molecules which lower surface tension and prevent droplet flocculation by absorption on the droplet surfaces and form a protective coating around the droplets which prevents them from coalescing with each other (McClements, 1999; Krstonosic, et. al 2009).

For all emulsions, emulsifier concentration had a considerable effect on the viscosity. The viscosity of the system was observed to increase with increasing hydrocolloid concentration. Further, the apparent viscosity of stabilized emulsions only increased slightly with protein concentration.

A primary role of an emulsifier is to migrate to the interface of the newly formed droplets, form a protective layer which prevents aggregation,

and reduce the interfacial tension, therefore stabilizing against coalescence (McClements, 2005).

Particle size of the oil phase and their size distribution play an important role in evaluating emulsion (McClements, 1999). The stability of an emulsion to gravitational separation can be enhanced by reducing the droplet size (McClements, 1999). In general large droplet tend to coalesce faster than small ones (Bergenstahl, and Claesson, 1990), emulsion stability is a measure of the rate at which emulsion creams, flocculates or coalesces. The rate of these changes can be measured by determining the size and distribution of the oil droplets in the emulsion.

Several possible methods for emulsion formation and a wide range of equipment are available for emulsion formation. These methods include shaking, stability stirring, and injection, and the use of colloid mills, ultrasonic, and homogenizers. The later are the most common equipment used in laboratories for emulsion formation (Walstra, 1983).

The stability of an oil-in-water emulsion is influenced by many factors, including the composition and physicochemical properties of both the oil and aqueous phase (Phipps, 1985; Walstra and Smulder, 1998). The aqueous phase of an emulsion may contain a wide variety of components, including minerals, acids, bases, biopolymers, sugars, alcohols, and gas bubbles. Many of these components will alter the size of the droplets produced during homogenization because of their influence on rheology, interfacial tension, coalescence stability, or adsorption kinetics (McClements, 2005; Saifullah, 2011). In many food emulsions, droplet coalescence may be promoted by the presence of solid particles or crystals due to their ability to disrupt the thin film separating the droplets, for example fat, ice, sugar, or salts crystals (McClements, 2005).

Chapter Two

Materials and Methods

2. Materials and Methods

2.1. Material, equipment and instrumentation

Dried gum samples were kindly provided by the supervisor, from gum research Centre, Sudan University of Science and Technology. The samples were ground into fine powder to pass 0.4 mm mesh screen. The prepared samples were kept in tight containers and stored at room temperature until used. D-lemonine oil from Sigma-Aldrich, and high quality ethylene glycol laboratory Grade from Carolina.

Laser light diffraction mastersizer (Malvern Instrument Limited, Worcestershire, UK), homogenizer (Ultra-turrax IKA T25 Basic, WERKE, Romania), turbidity meter (HANNA Instrument, HI 98713-01 Romania), Sensitive balance, and hot plate magnetic stirrer (Fisher-Scientific Isotemp.) were used for the experiment.

2.1.1. Gum solutions preparation

Gum concentrate (5% w/w) of each sample was prepared by dissolving 20.00 gm. of finely powdered Gum Arabic sample in 380.0 ml. distilled water by dispersing the fine powder on the distilled water under magnetic agitation on a hot plate at 40 °C for 1 hour. Then the gum solution was filtered through filtering cloth to remove undesirable impurities, these solutions were kept in closed vessels as stock solutions which ready for mixing with other ingredients before homogenization. The resultant solutions were used to prepare a set of 4%, 3%, 2%, and 1% (w/w) gum solutions by making appropriate dilutions with distilled water.

For varying protein concentrations, a stock solution of 2% (w/w) gum protein concentration was prepared, by dissolving an appropriate amount of gum sample in an appropriate amount of distilled water, following the same procedure described above, then solutions of 0.15%, 0.1%, and 0.05% (w/w) gum protein were prepared from the stock solution by successive dilution.

2.1.2. Emulsions preparation

Emulsions were prepared by blending D-lemonine oil and each of the gum concentrations (5%, 4%, 3%, 2%, and 1% w/w) and each of the gum protein concentrations (0.15%, 0.1%, and 0.05% w/w), using the homogenizer, at 19000 rpm for 5 min.

To 10.0ml(5%w/w) gum solution, in a clean suitable container, 0.25 gm of the oil phase and 0.1 ml of ethylene glycol were added, and then homogenized properly to form the emulsion. The same procedure was repeated for all other concentrations using constant amount of oil phase (0.25 gm) with 10.0 ml of gum solutions and following the same procedure for gum protein solutions.

The basic idea is to prepare emulsions using different *Acacia* gums and examine their stability against time. The emulsions were prepared using two sets of gum solutions. The first set consists of a series of solutions of gum varying in their gum concentration(5%, 4%, 3%, 2%, and 1% w/w). The second set shall be a series of gum solutions varying in their gum's protein content(0.15%, 0.1%, and 0.05% w/w). The following point was taken in consideration.

The content of protein in *Acacia Senegal* var *Senegal* gum(Hashab) has been established in the level of 2 % (w/w). The latest values of the protein content of Hashab even taken as an average of a set of published data (**Karamallah et. al., (1998)**).

The theme for preparation of the solutions sets of gum's solution having different concentrations are as shown below:

2.1.2.1. SET (1) Varying concentration of gum:

This is straight forward procedure, large quantity of 5% w/w gum solution as stock solution was prepared by dissolving 20 grams of gamin *380grams* grams of distilled water. The remaining members of the **SET(1)** are prepared from the 5% stock solution by making the appropriate dilutions with distilled water to form 4%, 3%, 2% and 1% solutions.

2.1.2.2. SET (2)Varying concentration of protein in the gum solutions:

For preparation set (2)solutions, the emphasis was on the concentration of gum's protein. As the protein content of Gum Arabic is taken as around 2%. Hence a gum solution having 0.2% (w/w) protein content was prepared as follows:

Since 10 grams of gum shall contain 0.2% of gum's protein, 10 grams of gum dissolved in 90 grams of water should give a gum solution containing 0.2% protein (w/w). It is also clear that the percentage of gum in this solution was 10 grams per 100 grams i.e. 10% gum concentration.90 grams of water was added to 10 g of gum to prepare a solution of protein concentration of 0.2% (stock solution). By successive dilution, solutions of 0.15%, 0.10% and 0.05% gum's protein were prepared.

The smallest oil droplet size in the emulsion can be achieved when the ratio of gum to oil is 2:1. Hence no stable emulsion can be produced when the ratio of the gum to oil is 0.1:1. Taking these facts in consideration it would be appropriate to the gum to oil ratio in the process of emulsion preparation was adjusted within the limits mentioned above.

The following procedure for preparation of emulsions was adopted to prepare **SET (1)**:

In a clean suitable container 10 mL of 5% gum solution was taken, and to this 0.25 grams of oil phase was added, and then homogenize properly to form an emulsion. The same procedure was repeated using constant amount of oil phase(0.25 g) with 10 mL of gum solutions of concentrations 4%, 3%,

2% and 1%. Only one emulsion was prepared and the turbidity measurements completed before moved to prepare the next emulsion.

Emulsions of SET(2) were prepared following the same procedure detailed above. All precaution mentioned above also applied here.

2.2. Measurements of emulsion stability

Turbidity measurements are used here. The following procedure was adopted: Immediately after preparing the emulsion. The dilute working emulsion solution was diluted, 1:1000 . Absorbance measurements shall be conducted on this dilute emulsion.

2.2.1. Stability measurements as % of separation

Background:

The emulsion stability have been studied extensively by many research groups and various methods of determining emulsion stability have been proposed such as droplet size analysis(Walstra, and Oortwijn, 1969), measuring physical properties of emulsion such as creaming and gravitational separation(Barry, 1968; Saifullah, 2011), accelerated test(Vold and Acevedo, 1977), and light scattering(Goulden, 1958).

To theoretically predict the rate at which separation of droplets by gravitation occurs in an emulsion, it is necessary to have information about the densities of the dispersed and continuous phase, the droplet size distribution, and the rheological properties of the continuous phase. However, the theoretical predictions are not enough to take into account the inherent complexity of food emulsions. For this reason, it is often necessary to directly measure the gravitational separation of the droplets in an emulsion.

The simple method of measuring gravitational separation is to place an emulsion in a transparent bottle, and leave it for certain period, and then measure the height of the interface between droplet-depleted and droplet-rich layers.

For stability studies by gravitational separation, immediately after emulsion preparation, 10 ml aliquots of each sample of the homogenized emulsions were transferred to graduated cylinders of 10ml volume. The total height of homogenized emulsion was measured with a measuring scale. During storage, at room temperature (25°C-27°C), the loss of height was observed and reading was taken on specific storage interval. The stability, as % of separation, was calculated as follows:

$$\% \text{Separation} = \left(\frac{H_1}{H_0} \right) \times 100 \dots\dots\dots (1)$$

Where H_0 represents the emulsion initial height and H_1 is the upper phase height.

Results of the visual observation is presented in table(3)

A sophisticated method of monitoring gravitational separation is to use light scattering. The light source which is a monochromatic beam of near infrared light is directed on an emulsion in a glass measurement tube. The percentage of transmitted and scattered light is measured as a function of emulsion height by scanning the light beam up and down the sample using a stepper motor. The variation in droplet concentration with emulsion height can then be deduced from the percentage of transmitted and scattered light using a suitable theory or calibration curve. The technique could be used to measure both the size and concentration of the droplets at any height by measuring the angular dependence of the intensity of the scattered light.

2.2.2. Stability Index Calculations

In each the clean test tubes Exactly 9 mL of distilled water was placed.

Then to tube **No.1** exactly ONE mL of the emulsion, 5% w/w gum solution as stock solution used for preparation of SET1 solutions, was added (using a pipette), shake thoroughly to mix its content perfectly. Tube **No.1** was let to stand for one minute. Using pipette exactly ONE mL from the diluted emulsion in tube **No.1** was withdrawn and introduced into tube **No.2**

and mixed thoroughly. Tube **No.2** was allowed to stand for a minute then exactly one ml from it withdrawn and placed into tube **No. 3**. Mixed thoroughly and the content allowed to stand for one minute. This final solution in tube **No.3** was 1:1000 dilute compared to the original emulsion. Immediately the turbidity was measured(**T₁**). Then after **one hour** the turbidity was measured again the absorbance (**T₂**). (Karamalla, et.al, 1998.)

The stability index is calculated as follows:

$$SI = [T_2] / [T_1] \dots\dots\dots (2)$$

Where SI, the Stability Index, T₁, the turbidity at zero time, and T₂, the turbidity after one hour

The same procedure is exactly repeated for the other concentration from SET1 and SET2.

In set1 gum: oil ratio is the controlling factor while in set2 gum's Protein: oil ratio is the controlling factor

2.2.3. Turbidity measurements

Turbidity measurements were used to determine emulsion stability and they provide a faster approach to evaluate emulsion stability.

Dilute working emulsion solution was prepared (1:1000 dilute compared to original emulsion) immediately after emulsion preparation. Turbidity measurements were conducted on this dilute emulsion using turbidity meter. A clean, screw capped cuvet, filled with 10 ml dilute, working solution, and then taken to the turbidity meter, the instrument was carefully calibrated with formazin standards. The results were reported in nephelometric units (NTU).The first reading was taken at zero time, and the next readings were taken at intervals of 24 hours. Accuracy of the instrument, as specified by the manufacturer and based on instrument calibration, is approximately ± 0.01NTU.All readings were repeated as triplicates, with good agreements being found among readings. Emulsion stability was calculated as follows:

$$\textit{Emulsifying stability (ES)} = \frac{\textit{First reading at zero time..... (3)}}{\textit{Reading at (x) time}}$$

2.2.4.Emulsion / Average Droplet Size

The average droplet size of the emulsions was measured using a Malvern Mastersizer 2000 particle size analyzer. This instrument measures the intensity of laser light scattered from dilute emulsion samples and reports the particle size distribution that gives the best fit between theoretical(Mie theory) and experimental values of intensity versus scattering angle [Charoen, et.al., 2011]. To avoid multiple scattering effects, the measurements were conducted by adding the concentrated emulsions drop wise Into the sample dispersion unit until an obscuration value of between 1% and 2% was reached. The average droplet size measurements were reported as volume-weighted means, D [McGorin, et.al, 2007] or also known as the De Brouckere mean diameter. The measurements of the droplet size were performed immediately after preparation and were reported as the average of two separate measurements, with five readings made for each measurement.

Droplet size distribution of the emulsions was analyzed using a Mastersizer 2000 laser diffractometer (Malvern Instruments, UK). The Mastersizer 2000 measure mean particle diameters in the range of 0.02 to 2000 μm . Distilled water was used as dispersion medium. A small sample of emulsion was suspended in water under agitation, and the dropletsize distribution was monitored during each measurement until successive readings became constant. The droplet size of the emulsion was described by the volume median diameter (VMD). The ratio of the particle of 1 μm or more and 2 μm or more were also calculated.

Results of droplet size measurements are presented in figures (10-14)

2.2.5.Emulsion long-term stability test / Accelerated temperature stress test

In this experiment, the long-term stability of the emulsion of *Acacia Senegal* samples was evaluated by using the accelerated stress at 60C°. The particle size of the stored emulsion at 60 C° was measured as described above Mastersizer 2000, the results illustrated in figure (12) at given time intervals (3 days and 7days).

Results of emulsion long-term stability test / Accelerated temperature stress test are presented in figures (11-14)

2.3. Statistical methods

Measurements were conducted in three replicates using same samples. The average and standard deviation values were calculated from these replicate measurements.

Chapter Three

Results and Discussion

Results and Discussion

3.1. Stability measurements as % of separation

The % of separation, droplets mean diameter (d) of the emulsions prepared with different concentrations of gum and D- lemonine oil

Gum Type	Separation (%)	d (µm)
<i>A. Mellefera</i>	0.5	0.25
<i>A. Seyal var Seyal</i>	2	0.6
<i>A. Senega var Senegall</i>	10	0.6
<i>A. Tortilis var radiana</i>	12	0.5

Table 3.1: Properties of the emulsions produced with different gum concentrations and D- lemonine oil.

Emulsion destabilization process is evaluated by visual observation in a 7-day storage study. The stability of emulsions, prepared with the gums, during a 7-day storage study at 25°C, indicated that emulsion stability was greater in *A.Mellefera* than other gum samples, at the 7th day of storage. The rate of sedimentation, creaming and particle size changes varied between gums stabilized emulsions according to gum type and gum viscosity

3.2. Stability Index

Concentration	Stability Index			
	A. Senegal	A. Seyal	A.Tortilis	A. Mellefera
1%	0.52	0.60	0.55	0.62
2 %	0.57	0.68	0.58	0.63
3 %	0.66	0.70	0.61	0.65
4 %	0.76	0.77	0.64	0.68
5 %	0.93	0.80	0.85	0.91

Table (3.2): Stability Indices of different *Acacia* gums at different gum concentrations

It is obvious that increasing turbidity with time is an indication of instability of the emulsion. The emulsion stability of the different gums as a function of gum concentration, was determined in term of physical stability, represented as stability index is shown in table (3.2) and represented in figure (3.1).

The degree of creaming, immediately after preparation of the emulsions of different *Acacia* gums at different concentrations, was studied. The results indicate that, as expected, higher gum concentration reduced the extent of creaming and increased the emulsion stability. The emulsion with 5% *Acacia senegal* and *Acacia mellefera* resulted in emulsions with the highest physical stability, with stability index over 90% within the 60 min. shelf storage period. The emulsion with 5% gum concentration of other gums resulted in emulsions with stability index over 80% within the same storage period.

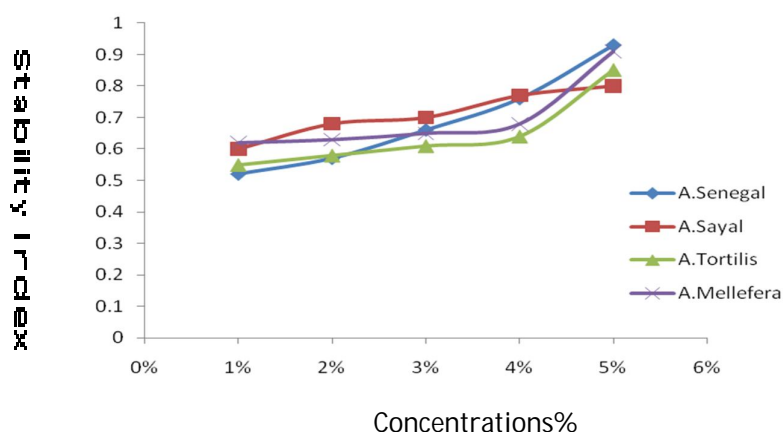


Figure (3.1) Stability Indices of different *Acacia* gums at different concentrations

3.3. Emulsion Stability

Turbidity method has been used to determine emulsion stability (Song, et.al. 2000). There are many factors that affect emulsion properties such as homogenization condition, proportion of emulsion component ...etc.

3.3.1. Effect of Gum Concentration

The average droplet size and interfacial surface area of the various emulsions as established from the turbidity data was found to vary according to the initial gum arabic concentration. During the emulsification process the homogenization produces droplets which have a certain interfacial surface area and the value will vary according to the type of homogenizer and the conditions used. If there are sufficient gum arabic molecules present in solution during homogenization to adsorb at the oil-water interface and fully coat the droplets, coalescence will be prevented. If, however, there are insufficient gum Arabic molecules present, coalescence will take place until the interfacial surface area is reduced to a value such that full coverage of the droplets can be achieved. Practically, emulsifier adsorbs to the surface of the droplets during homogenization, by forming a protective membrane which prevent them from coming close enough together to coalesce (Walstra, 1983.; Walstra, 1996a). Droplets formed with gum concentration below 4%, revealed that the gum concentration might be too low to form a protective membrane to prevent the droplets to come close.

Turbidity measurements for emulsion stabilized by *A. senegal* are . Figure (3.2) plots the emulsion stability of *Acacia senegal* at different concentration, the stability is clearly a function of time and gum concentration. As shown in Figure (3.2), 5% and 4% gum concentration showed better stability than that of emulsion containing lower% gum concentration.

The stability drops sharply within the first 3 days, and after 3 days shelf storage, the stability drops slightly and approached a plateau, the emulsion containing gum concentrations lower than 4% showed lower stability than emulsions containing 5% and 4% gum concentration. The later concentration seems to be the optimum concentration of *Acacia senegal* var. *Senegal* gum to form a stable emulsion over the selected period of time.

No_	Time (hrs)	5%		4%		3%		2%		1%	
		(NTU)	ES	(NTU)	ES	(NTU)	ES	(NTU)	ES	(NTU)	ES
1	0	13.9	1.00	10.1	1.00	15.0	1.00	8.57	1.00	7.47	1.00
2	24	11.7	0.84	5.83	0.58	9.61	0.64	6.80	0.79	3.99	0.53
3	48	7.01	0.50	4.52	0.45	6.06	0.40	3.29	0.38	2.67	0.36
4	72	5.59	0.40	4.35	0.43	4.09	0.27	2.96	0.35	2.58	0.35
5	96	5.15	0.37	4.28	0.42	3.79	0.25	2.52	0.29	1.99	0.27

Table(3.3): Emulsion Stability of *Acacia senegal var Senegal*- different concentration

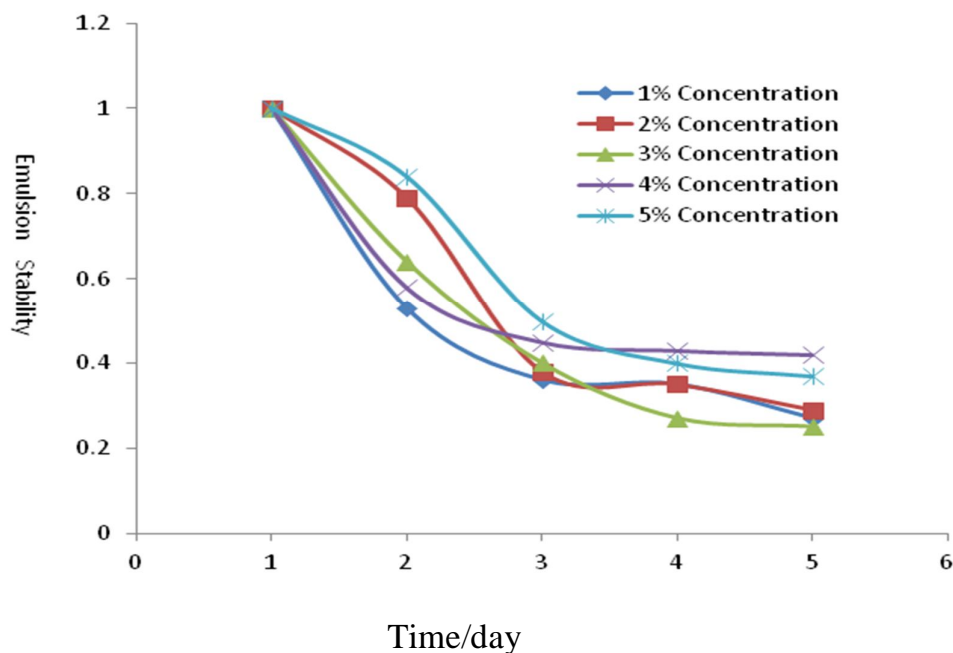


Figure (3.2) Emulsion Stability of *Acacia Senegal var Senegal*- different concentration

sharply within the first 2 days, after which the stability values approached a plateau. The stability of emulsion containing 4% gum concentration exhibited better stability than other gum concentration similar to *Acacia Senegal*. Instability started just after the 1st day of formulation of emulsion, in the case of 4% gum concentration, after the 2nd day of shelf storage, the stability values approached a plateau, while the stability values for other concentrations approached a plateau after the 3rd day of shelf

storage. The 4% gum concentration seems to be the optimum concentrations of *Acacia seyal* to form an stable emulsion over the selected period of time.

No_	Time (hrs)	5%		4%		3%		2%		1%	
		(NTU)	ES	(NTU)	ES	(NTU)	ES	(NTU)	ES	(NTU)	ES
1	0	16.3	1.00	10.2	1.00	11.5	1.00	8.36	1.00	7.83	1.00
2	24	12.0	0.74	8.32	0.82	10.3	0.90	5.33	0.64	5.06	0.65
3	48	5.63	0.35	3.94	0.39	5.40	0.45	2.87	0.34	3.50	0.45
4	72	4.26	0.26	3.67	0.36	4.08	0.35	2.40	0.29	2.75	0.35
5	96	4.06	0.25	3.36	0.33	3.65	0.32	2.37	0.28	2.69	0.34

Table(3.4): Emulsion Stability of *Acacia seyal* var. *Seayl*- different concentration

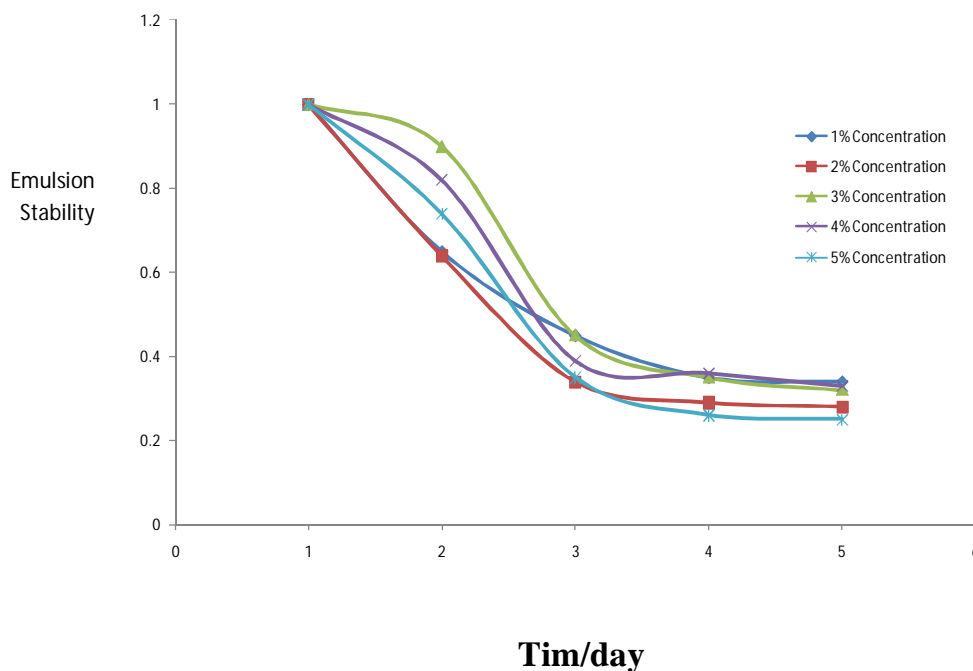


Figure (3.3) Emulsion Stability of *Acacia seyal*- different concentratin

Figure (3.4) shows that stability of emulsion system containing *Acacia tortilis* at different concentrations, the results reflect the dependence of emulsion stability on gum concentration. Higher gum concentration(5%)

gives the most stable emulsion. As shown in the above results, stability drops sharply within the first 2 days for the lower concentration, after which the stability values approached a plateau. In emulsions containing 4% and 5% gum concentration stability drops gradually up to the third day of shelf storage, after which the stability values approached a plateau. These results are in good agreement with the findings of many authors (EriAkiyama et al. 2005); Song, et al. 2000)

No_	Time (hrs)	5%		4%		3%		2%			
		(NTU)	ES	(NTU)	ES	(NTU)	ES	(NTU)	ES	(NTU)	ES
1	0	12.1	1.00	10.9	1.00	10.3	1.00	11.0	1.00	12.3	1.00
2	24	6.27	0.52	7.08	0.65	5.05	0.49	6.71	0.61	4.64	0.38
3	48	4.42	0.37	3.97	0.36	3.09	0.30	3.21	0.29	2.97	0.24
4	72	3.61	0.30	3.29	0.30	2.80	0.27	2.99	0.27	2.26	0.18
5	96	3.13	0.26	2.97	0.27	2.44	0.24	2.27	0.21	1.36	0.11

Table(3.5)) Emulsion Stability of *Acacia Tortilis* var. *radiana* different

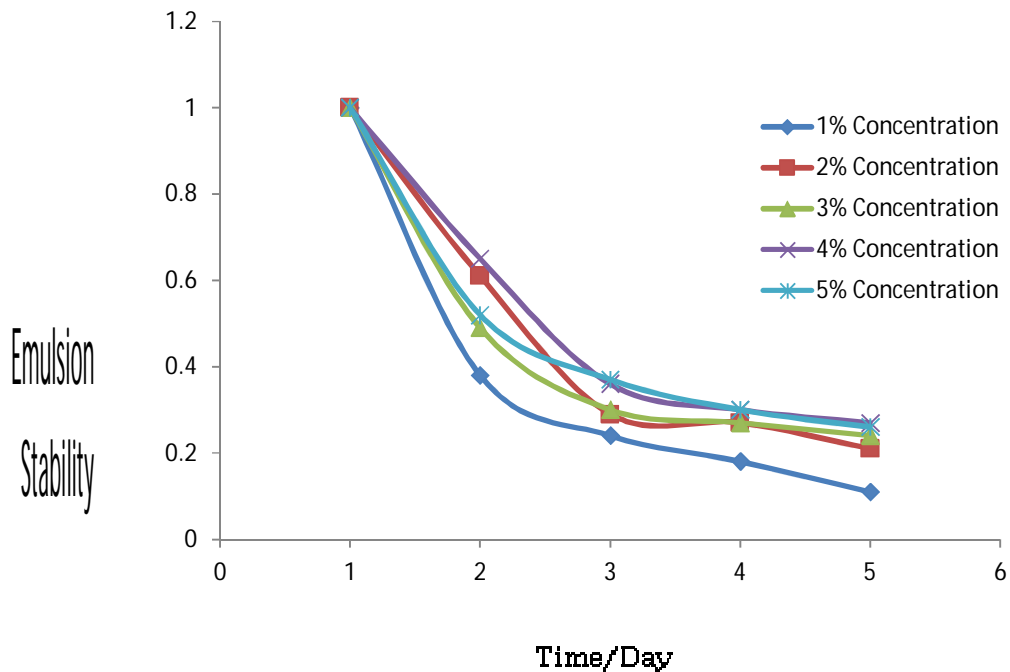
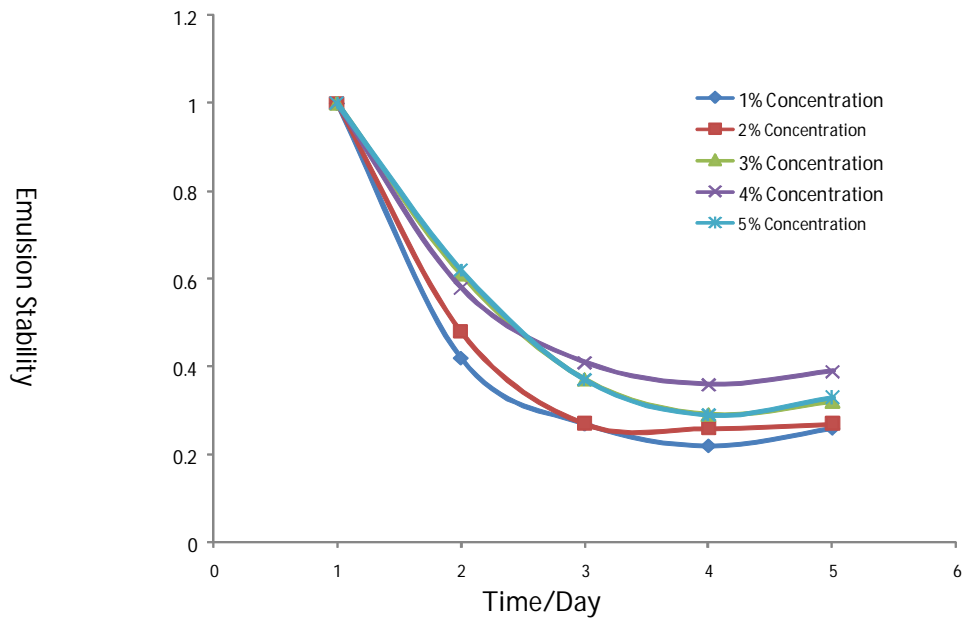


Figure (3.4) Emulsion stability of *Acacia Tortilis* var. *radiana* - different concentration

No_	Time (hrs)	5%		4%		3%		2%		1%	
		(NT U)	ES	(NTU)	ES	(NT U)	ES	(NT U)	ES	(NT U)	ES
1	0	12.2	1.00	7.25	1.00	13.8	1.00	11.9	1.00	9.36	1.00
2	24	7.62	0.62	4.21	0.58	8.35	0.61	5.74	0.48	3.92	0.42
3	48	4.49	0.37	2.98	0.41	5.16	0.37	3.18	0.27	2.52	0.27
4	72	3.53	0.29	2.62	0.36	4.02	0.29	3.14	0.26	2.08	0.22
5	96	4.07	0.33	2.84	0.39	4.37	0.32	3.22	0.27	2.46	0.26

Table(3.7) Emulsion Stability of *Acacia Mellefera*- different concentration



Figure(3.5) Emulsion stability of *Acacia Mellefera*- different concentration

3.3.2. Effect of Protein Concentration

3.3.2.1. Stability Indices

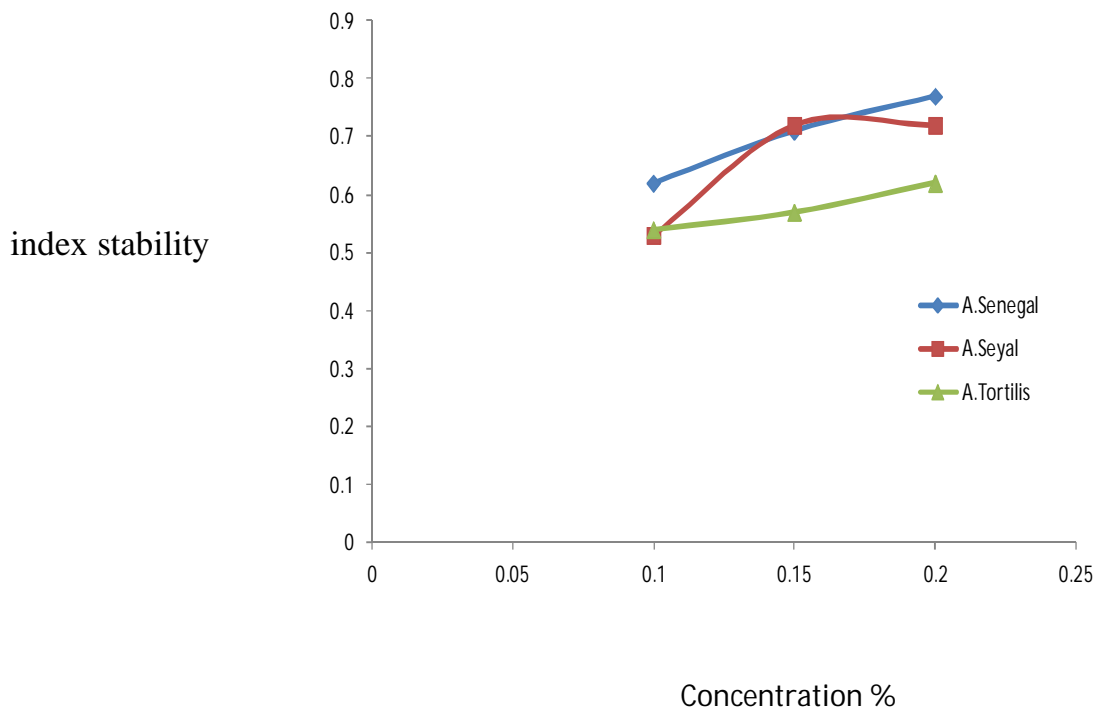
The emulsion stability of the different gums as a function of protein concentration, determined in term of physical stability, represented as the stability index .

The degree of creaming, immediately after preparation of the emulsions of different *Acacia* gums at different protein concentrations, was studied. Results indicate that, as expected, higher gum concentration reduced the extent of creaming and increased emulsion stability. The emulsion with 2% protein content of *Acacia senegal var. Senegal* resulted in emulsions with the highest physical stability, with stability index over 75% within the 60 min. shelf storage period. The emulsion with 2% gum protein concentration of other gums resulted in emulsions with stability index over 60% within the same storage period.

Acacia Mellefera was excluded , it's very high viscosity was not enable emulsions preparation of comparable concentrations.

Concentration	stability Index		
	<i>A. senegal</i>	<i>A. seyal</i>	<i>A.Tortilis</i>
0.1%	0.62	0.53	0.54
0.15 %	0.71	0.72	0.57
0.2 %	0.77	0.72	0.6

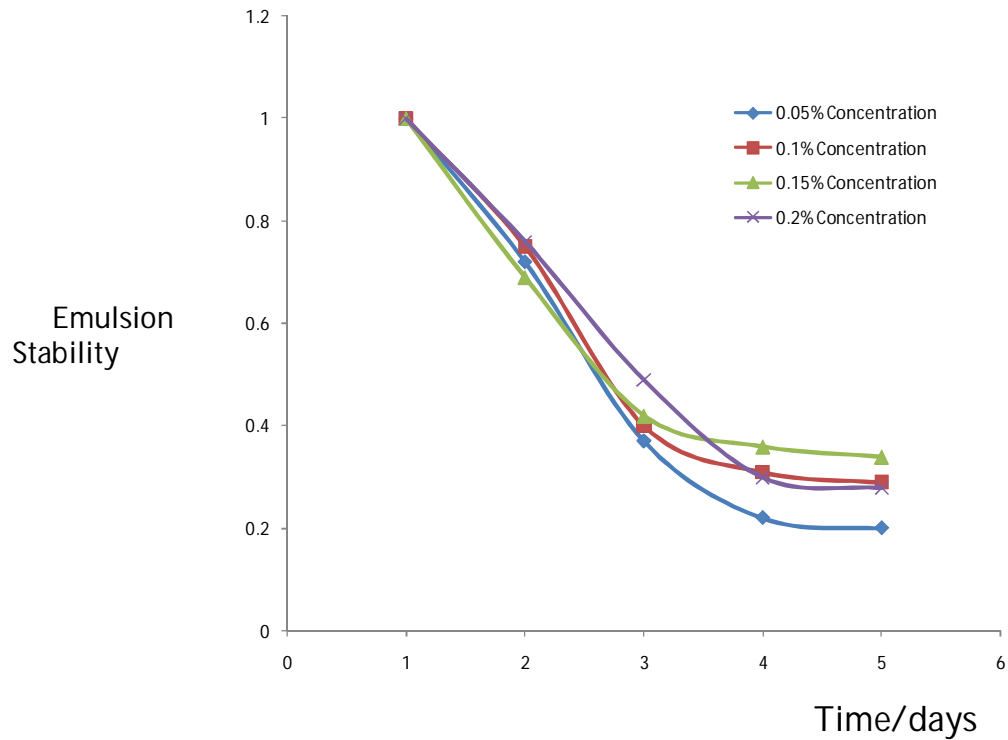
Table(3.8) Stability Indices of different *Acacia* gums at different protein concentration



The effect of protein concentration of differentiated *Acacia* gums on the emulsion stability (3.6, 3.7, 3.8 and 8). The stability which is, clearly, a function of time and protein concentration, drops sharply within the first three days shelf storage period, after which the stability values approached a plateau. For *Acacia* gums, these results, in general, indicate that, higher protein concentrations result in the highest emulsion stability (ES is 60% up to the 5th day for 0.2% protein concentration). These results are in good agreement with the findings of Randall et. al., (1988), who concluded that Gum Arabic, which was reported to contain < 2% protein, is responsible for the emulsifying properties of the gum as a whole.

No_	Time (hrs)	0.2%		0.15%		0.1%		0.05%	
		(NT U)	ES	(NT U)	ES	(NT U)	ES	(NT U)	ES
1	0	19.6	1.00	12.5	1.00	12.9	1.00	17.9	1.00
2	24	14.8	0.76	8.57	0.69	9.69	0.75	12.9	0.72
3	48	9.65	0.49	5.30	0.42	5.22	0.40	6.66	0.37
4	72	5.87	0.30	4.49	0.36	3.97	0.31	3.95	0.22
5	96	5.43	0.28	4.31	0.34	3.75	0.29	3.52	0.20

Table(3.10) Emulsion Stability of *Acacia seyal* var. *Seyal*- different protein concentration

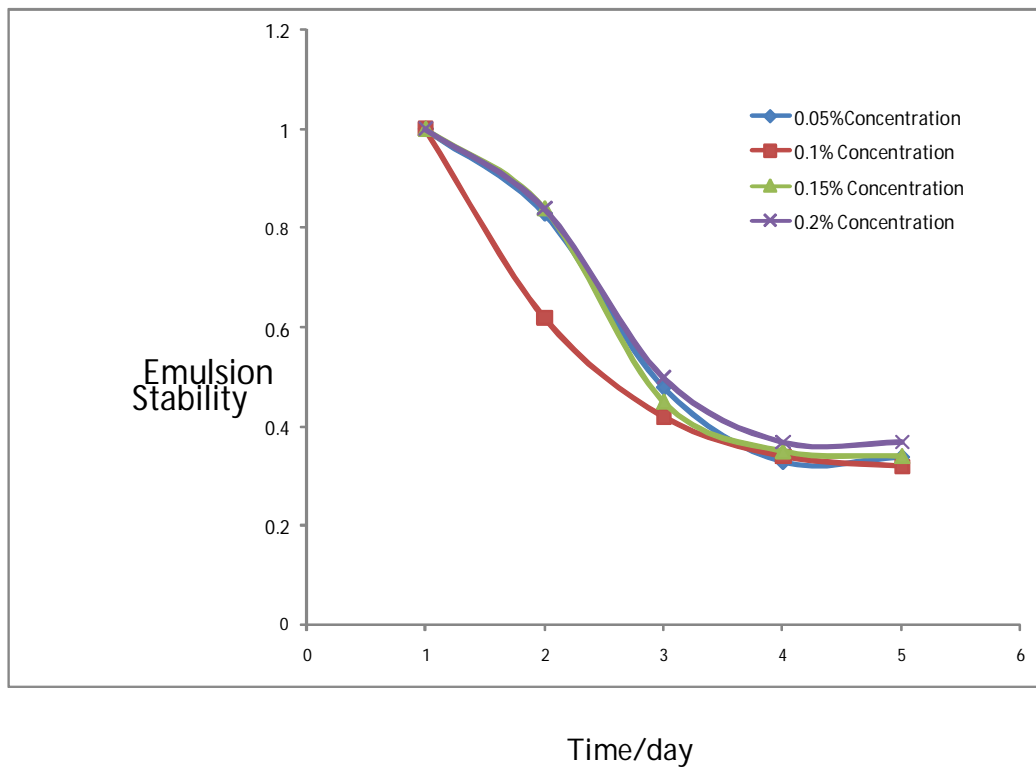


Figure(3.8) Emulsion Stability of *Acacia seyal* var. *Seyal*- different protein concentration

No_	Time (hrs)	0.2%		0.15%		0.1%		0.05%	
		(NT U)	ES	(NT U)	ES	(NT U)	ES	(NT U)	ES
1	0	10.8	1.00	10.8	1.00	9.49	1.00	9.42	1.00
2	24	9.09	0.84	9.11	0.84	5.84	0.62	7.80	0.83
3	48	5.37	0.50	4.84	0.45	3.99	0.42	4.54	0.48
4	72	3.95	0.37	3.78	0.35	3.23	0.34	3.11	0.33
5	96	4.03	0.37	3.68	0.34	3.05	0.32	3.21	0.34

Table (3.11)

Emulsion Stability of *Acacia Tortelis* var *radiana*- different protein concentration



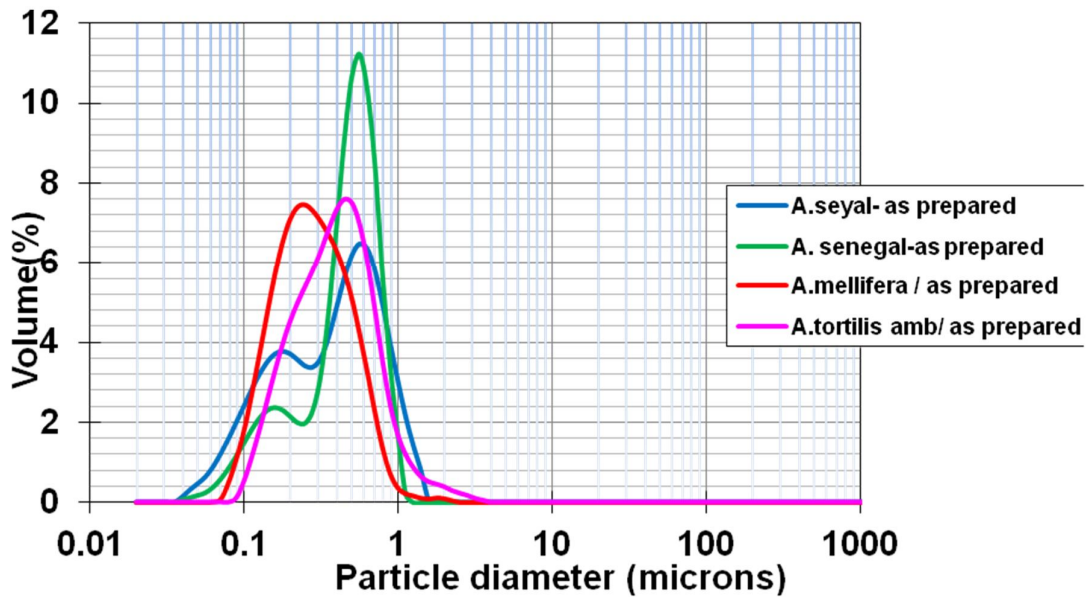
Figure(3.9): Emulsion Stability of *Acacia Tortelis*- different protein concentration

4.4. Mean droplet diameter

Mean droplet size (d) profile of all the emulsions studied are shown in Figures (9)

A monomodal distribution of droplet size with a small average diameter commonly signifies a stable system (Khalloufi, et.al. 2009.), as it is shown in Figure (3.10) all curves are monomodal. *A. Tortilis* var. *radiana* has an extent distribution of particle size from 0.09 to 5 μm and (d) 0.5 μm , *A. mellefera* has an extent distribution of particle size from 0.07 to 2 μm and (d) 0.25 μm , *A. seyal* has an extent distribution of particle size from 0.04 to 1.5 μm and (d) 0.6 μm , *A. senegal* has an extent distribution of particle size from 0.04 to 1.25 μm and d 0.6 μm .

The first observation from figure (3.10) is that all the emulsions are stable as one might expect from emulsions prepared with relatively high emulsifier-to-oil ratio. Curves for *A. senegal* and *A. seyal* with $d = 0.6 \mu\text{m}$ at a limited range of size distribution (0.04 to 1.5 μm) which refers to the emulsifying/stabilizing ability of these gums.

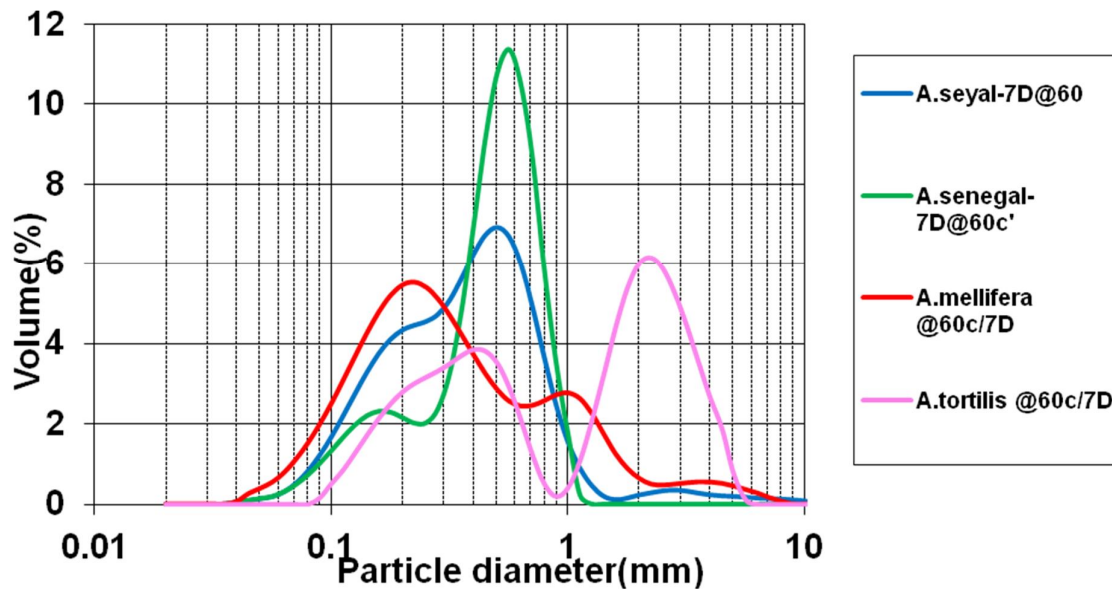


Figure(3.10) droplet size distribution of D-lemonine oil emulsions with different *Acacia* gums at zero time

3.5.Emulsion long-term stability test / Accelerated temperature stress test

Acacia gums emulsion stability was evaluated by using the accelerated stress at 60 °C. The particle size for the stored emulsion at 60 °C was measured by a Mastersizer 2000 ‘illustrated in figure (3.11) at different time intervals (3 days and 7days).

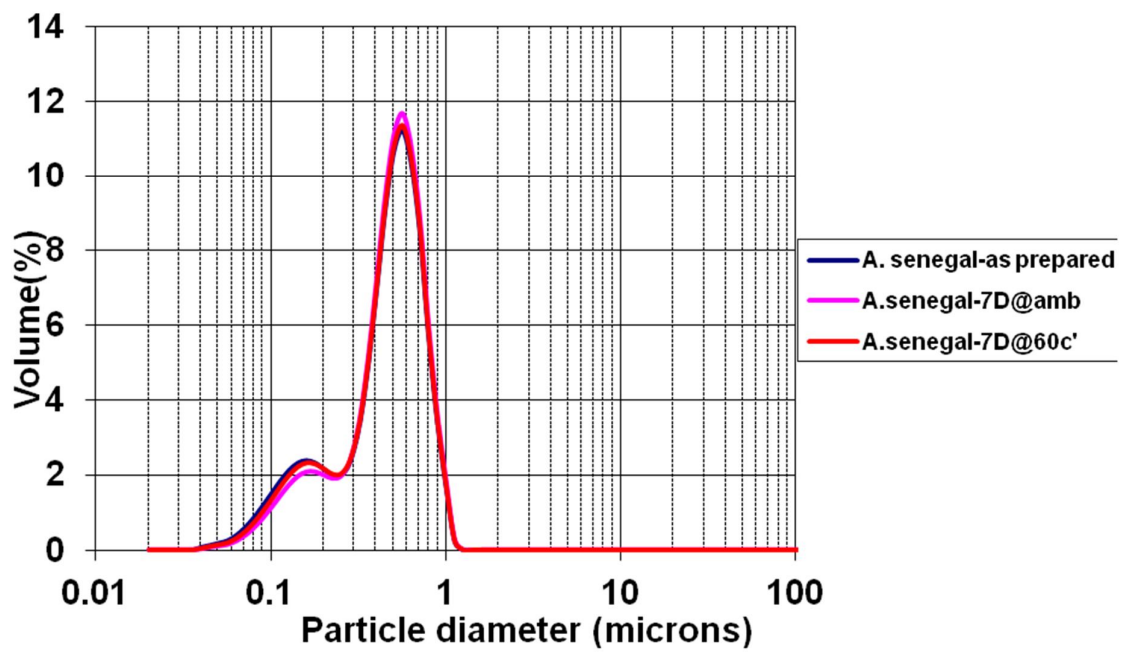
Figure(3.11) shows droplet size distribution of D-lemonine oil emulsions with different *Acacia* gums after storage for 7 days at 60 °C. The loss of height in the main peaks of the distribution function(V%) and the appearance of second peaks at larger droplet diameter d, indicates loss of stability over the observational time-scale via Oswald ripening, where the rate and nature of the loss of height and second peak is dependent on the type of gum.



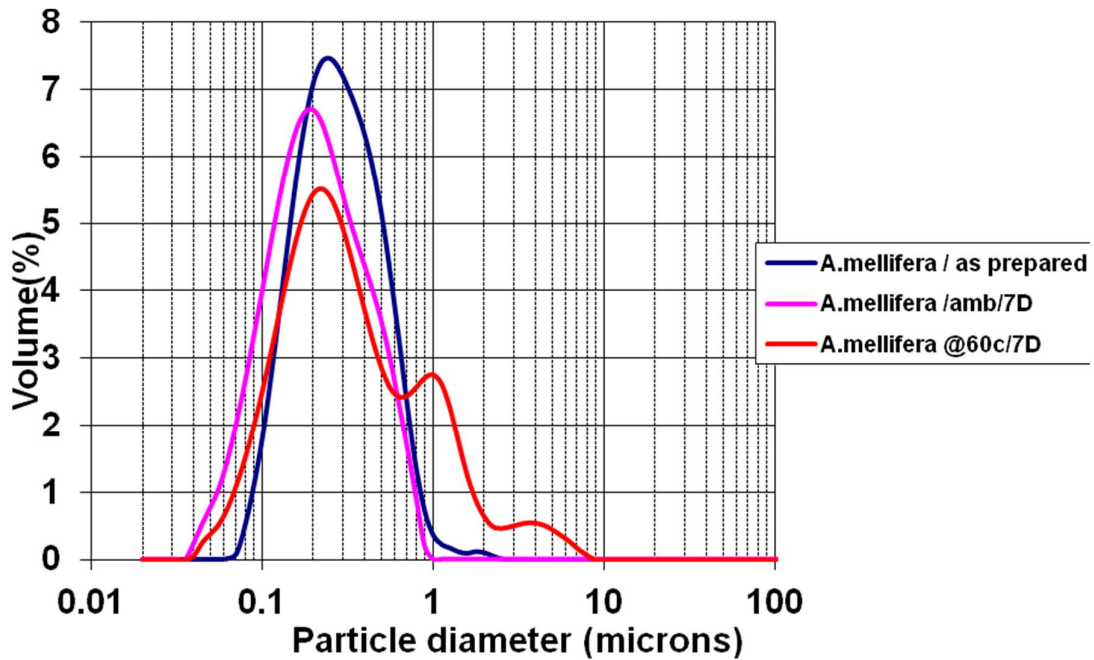
Figure(3.11) droplet size distribution of D-lemonine oil emulsions with different *Acacia* gums after storage for 7 days at 60 °C

Figure(3.12 and 3.13) shows comparison of droplet size distribution of D-lemonine oil emulsions with different acacia gums at storage conditions time of 7 days at 60 °C) the loss of height in the main peaks of the distribution function(V%) and the appearance of second peaks at larger droplet diameter d, indication of loss of stability over the observational time-scale via Oswald ripening, where the rate and nature of the loss of height and second peak is dependent on the type of gum.

The distribution curve on day 1 is relatively narrow, however, as time increases, the distributions curve becomes wider with a slight shift towards the right(Figure 10 and 11), indicating that the droplet diameter increased and also that diameter size became less homogenous.

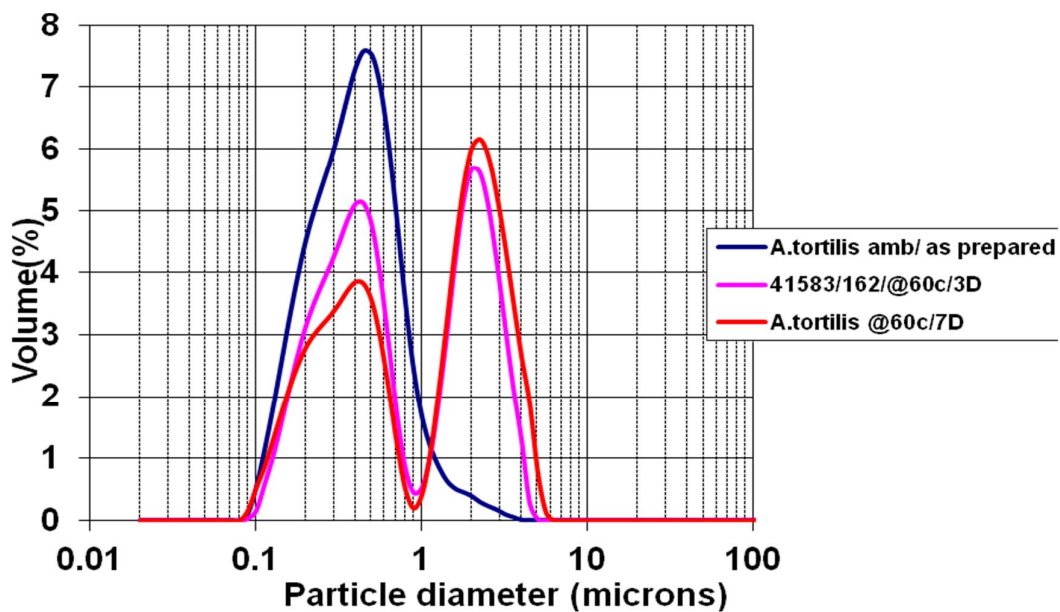


Figure(3.12) droplet size distribution of D-lemonine oil emulsions with *Acacia senegal* after storage for 7 days at 60 °C.



Figure(3.13) droplet size distribution of D-lemonine oil emulsions with *Acacia Mellifera* after storage for 7 days at 60 °C.

Figure(3.12 and 3.13) shows compare of droplet size distribution of D-lemonine oil emulsions with *Acacia senegal* var. *Senegal* and *Acacia Mellifera* at different storage conditions (for 7 days at ambient and 60 °C) the loss of height in the main peaks of the distribution function(V%) and the appearance of second peaks at larger droplet diameter d, are also apparent and indicate loss of stability over the observational time-scale via Oswald ripening., where the rate and nature of the loss of height and second peak is dependent on the type of gum.



Figure(3.14) droplet size distribution of D-lemonine oil emulsions with *Acacia Tortilis* after storage for 7 days and 3 days at 60 °C

Figure(3.14) shows droplet size distribution of D-lemonine oil emulsions with *Acacia Tortilis* var.*radiana* at different storage conditions (at 3 and 7 days at 60 °C) the loss of height in the main peaks of the distribution function(V%) and the appearance of second peaks at larger droplet diameter (d), indicated loss of stability over the observed time-scale via Oswald ripening, where the rate and nature of the loss of height and second peak is dependent on the type of gum and storage conditions .

The distribution curve on the first day is relatively higher, however, as time increases, the height of the distributions curve becomes lower with a slight shift towards the right, indicating that the droplet diameter increased and also that diameter size became less homogenous, where two groups of particle size are clearly state to be distinguished.

3.5. Conclusion

The results indicate that

- Varying the concentration of Acacia gum play a significant role in ES.
- Additions of Acacia gum of high protein concentration resulted in an increase in ES of the D-lemonine-in- gum solution emulsion, it is.
- Shelf-storage period was one of the most influential variable on ES.
- It has been found that Droplet diameter and droplet size distribution are key characteristic features of emulsion stability, contributing greatly to evaluate the physical stability of emulsions.

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