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Research Article

EFFECT OF GUM CONCENTRATION AND GUM PROTEIN CONCENTRATION ON EMULSIFYING PROPERTIES OF SOME ACACIA GUMS

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ABSTRACT
The most important question to be answered in this study is to demonstrate weather the emulsifying property of acacia gums under investigation, is due solely to the protein content of the gum or to the whole molecular component present in the gum. In this study d-lemonine oil flavor emulsions were prepared, by, homogenization method, with acacia senegal, acacia seyal, acacia tortilis and acacia mellifera . Different gum concentrations (1%, 2%, 3%, 4% and 5%) and different concentrations of gum protein (0.05%, 0.1%, 0.15% and 0.2%) were used for emulsion preparation. The observed emulsion stabilities were graphically represented from turbidity measurements, and droplet size distribution of the emulsion systems were also investigated by laser diffraction method. The results indicated that using different types of gums and different concentrations resulted in significant differences in emulsion stability (ES). It has been found that differences in ES may be ascribed to the differences in protein content. At 5% gum concentration and 0.2% protein concentration, acacia senegal EAS found to be the best emulsifier for d-lemonine and showed the most stable emulsion compared to other gums.

***Keywords*:** *Acacia Gum, D-Lemonine Homogenization, Emulsion Stability, Droplet Size*

INTRODUCTION
Gum arabic (GA) is a natural food additive. The most important application of (GA) have been used as an emulsifier in food and pharmaceutical industries. (GA) is considered to be the best type of gum, which can be used in dilute oil-in-water emulsion systems (Garti, 1999). One important example is its use as emulsifier/stabilizer for citrus oils as flavoring agents in soft drinks where the oils are converted into a water-dispersible emulsion (Verbeken *et al.,* 2003). (GA) is defined as the dried gummy exudates from the park of the stem and branches of certain varieties of acacia tree, such as *A. Senegal, A.Seyal, A.Tortilis* and other related african species (Tan, 1990). (GA) is colorless, tasteless and odorless (Mcclements, 2005); Chemically, (GA) is a complex mixture of macromolecules of different size and composition (mainly, carbohydrates and proteins). The gum consist of three distinct fractions: a high molecular mass arabinogalactan-protein complex (AGP) containing most of the total protein, a glycoprotein (GP)containing HTE rest of the protein, and a lower molecular mass fraction, an arabinogalactan polysaccharide (AGP), which is protein deficient. The gum has a variable protein content depending on the species (Anderson, 1986). The (AGP) fraction is mostly responsible for the emulsifying property 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.
Protein and oil is the most important ingredient in food emulsion.oil dispersions, which is in the form of small spherical droplet 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 the surface tension and prevent oil droplet from coming close enough together to aggregate (Dickinson and Golding, 1977; Paraskevopoulou *et al.*, 2007).
Studies by several groups (Randall *et al.,* 1988; Mertensand and Huyghebaert, 1988), indicate that the emulsifying and stabilizing property of gum is due to its protein-rich component (Garti, 1999), particularly the heterogenous AG-Protein, which the major component of (GA) (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-in-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 explain the ability of gum to act as the best emulsifier/stabilizer for oil-in-water emulsions (Yokoyama *et al.,* 1988; Randall *et al.,* 1988; Dong, 2012). 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, pharmaceuticals, encapsulation, food, etc. (Becher, 1985; Becher, 1988; Mcclements, 1999). Many food products in the market are in emulsion state, such as cheese, milk, salad dressings, beverages, coconut milk, soft drinks, syrup, gummy candies and creams (Mcclements, 1999; Verbeken *et al.*, 2003; Gonzalez, 1991).

In this paper, the emulsifying properties of four acacia gums, with approximately similar protein contents of c. 2%, were investigated.

The emulsions are thermodynamically unstable systems and have tendency to break down over time (Dickinson, 1992; Friberg and Larsson, 1997; Mcclements, 1999) and rapidly or slowly separate into two immiscible phases. The breakdown of an emulsion may be attributed to physiochemical mechanism 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. One of the main challenges of food product formulation is to increase shelf-life for a longer period of time, this can be achieved through the addition of emulsifier and stabilizer, which form a protective coating around the droplets prevent them from coalescing with each other (Mcclements, 1999; Mcclements, 2005; 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 increases slightly with protein concentration.

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 droplets tend to coalesce faster than the 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. These methods include shaking, stirring and injection, using colloid mills, ultrasonic and homogenizer. The later is 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 physiochemical 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 component, 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).

MATERIALS AND METHODS

Material, Equipment and Instrumentation

Dried gum samples were kindly provided by the supervisor, from gum research Centre, Sudan University of Science and Technology. Then 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 in this investigation. 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.

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 40^0Cfor 1 hours. 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 and 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.

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.

Stability Measurements as % of Separation

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).

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 (25^0C-27^0C), the loss of height was observed and reading was taken on specific storage interval. The stability, as % of separation, was calculated as follows:

$$\%Separation = \left(\frac{H1}{Ho}\right) X\ 100 \quad \text{..... (1)}$$

Where, *Ho* represents the emulsion initial height and $H_1$is the upper phase height.

Stability Index Calculations

In each of three clean numbered test tubes, exactly 9.0 ml distilled water was placed. Then to tube No.1, using micro pipette, an exactly one ml of the emulsion was added, then its content mixed well and allowed to stand for one min.

An exactly one ml withdrawn from tube No.1 and introduced into tube No.2, then its content mixed well and allowed to stand for one min. Then an exactly one ml withdrawn from it and introduced into tube No.3, then its content mixed well and allowed to stand for one min.

This final solution in tube No.3 was 1:1000 dilute compared to the original emulsion. Then the turbidity was measured immediately, T_1. Then after one hour the turbidity was measured again, T_2 (Karamalla *et al.*, 1998).

The stability index is calculated as follows:

$$SI = [T_2]/[T_1] \quad \text{..... (2)}$$

Where, SI, the Stability Index, T_1, the turbidity at zero time, andT_2, the turbidity after one hour

Turbidity Measurements

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

The turbidity of an emulsion containing oil droplet is a function of mean droplet size, concentration, and droplet size distribution, where the change in turbidity indicates changes in all these aspects. In an unstable emulsion, particle size and concentration change with time due to coalescence of the droplets.

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:

$$\text{Emulsifying Stability (ES)} = \frac{\text{First reading at zero time}}{\text{Reading at (x) time}} \quad \ldots\ldots (3)$$

Emulsion Droplet Size / 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, (McGorrin *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 droplet size 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 1µm or more and 2 µm or more were also calculated.

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 by a Mastersizer 2000, the results illustrated in figure 12 at given time intervals (3 days and 7 days).

Statistical Methods

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

RESULTS AND DISCUSSION

Kinetically stable emulsion is an emulsion which will not separate within a reasonable period of time, such good quality emulsion could be made by preventing the droplets formed during homogenization from merging together i.e. preventing them from coalescing with each other (McClements, 1999). This can be achieved by having a sufficient amount of emulsifier present during the homogenization process.

The emulsion stability can be expected to be higher when the droplet size is smaller.

An emulsion containing weighing agents and appropriate emulsifying components will result in kinetically high stable emulsion, if the average particle size of the emulsion is below 1 µm (Buffo and Reineccius, 2000).

In these investigations, to evaluate the effect of gum concentration on the stability of emulsion, five gum concentrations were used and the stability kinetics of emulsion was measured and plotted in Figures 2 - 5.

Emulsion Properties

The % of separation, droplets mean diameter (d) of the emulsions prepared with different concentrations of gum and D- lemonine oil are presented in Table 1.

Table 1: Properties of the Emulsions Produced with Different Gum Concentrations and D-Lemonine Oil

Gum Type	Separation (%)	d (µm)
A. Mellifera	0.5	0.25
A. Seyal	2	0.6
A. Senegal	10	0.6
A. Tortilis	12	0.5

The emulsion prepared with A. Mellifera showing very small phase separation, so it was the only kinetically stable one during the three days storage period, and this was in good agreement with the stability suggested by the smallest droplet size (0.25). All the other gum emulsions were unstable after the three days storage period, with a variable degree of instability. A.Seyal showed a small region of phase separation while A. Tortilis and A. Senegal showed the largest region of phase separations indicating very high kinetic instability under these experimental conditions.

Stability Index

The emulsion stability of the different gums was determined in term of physical stability, represented as stability index is shown in figure 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 Mellifera 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.

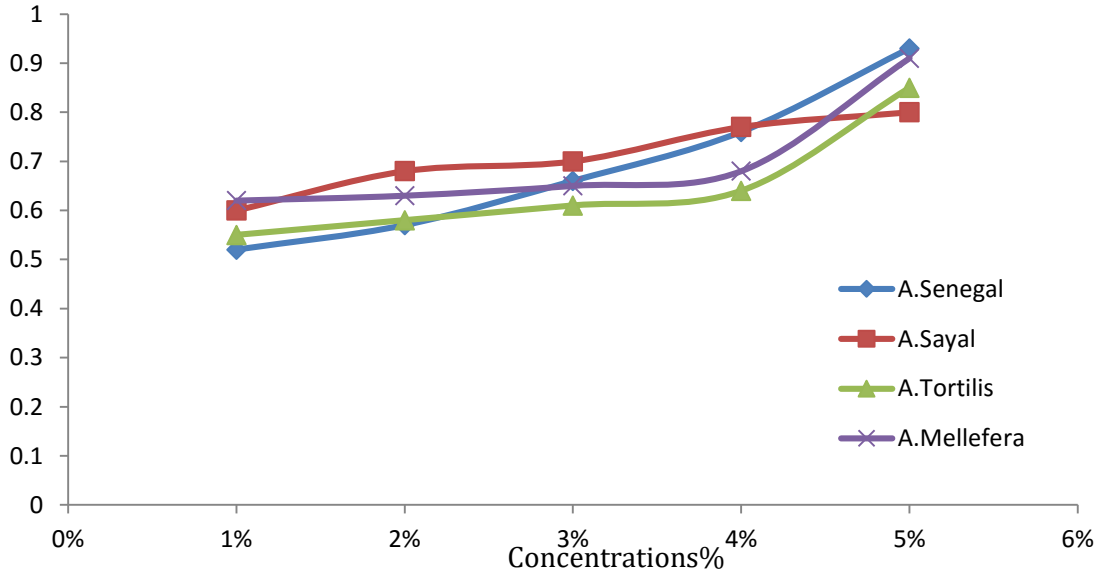

Figure 1: Stability Indices of Different Acacia Gums at Different Concentrations

Emulsion Stability

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

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.

Figure 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 2, 5% and 4% gum concentration showed better stability than that of emulsion containing lower% gum concentration. Just after 1st day instability exhibited both in the emulsion containing higher% and lower% gum.

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 to form a stable emulsion over the selected period of time.

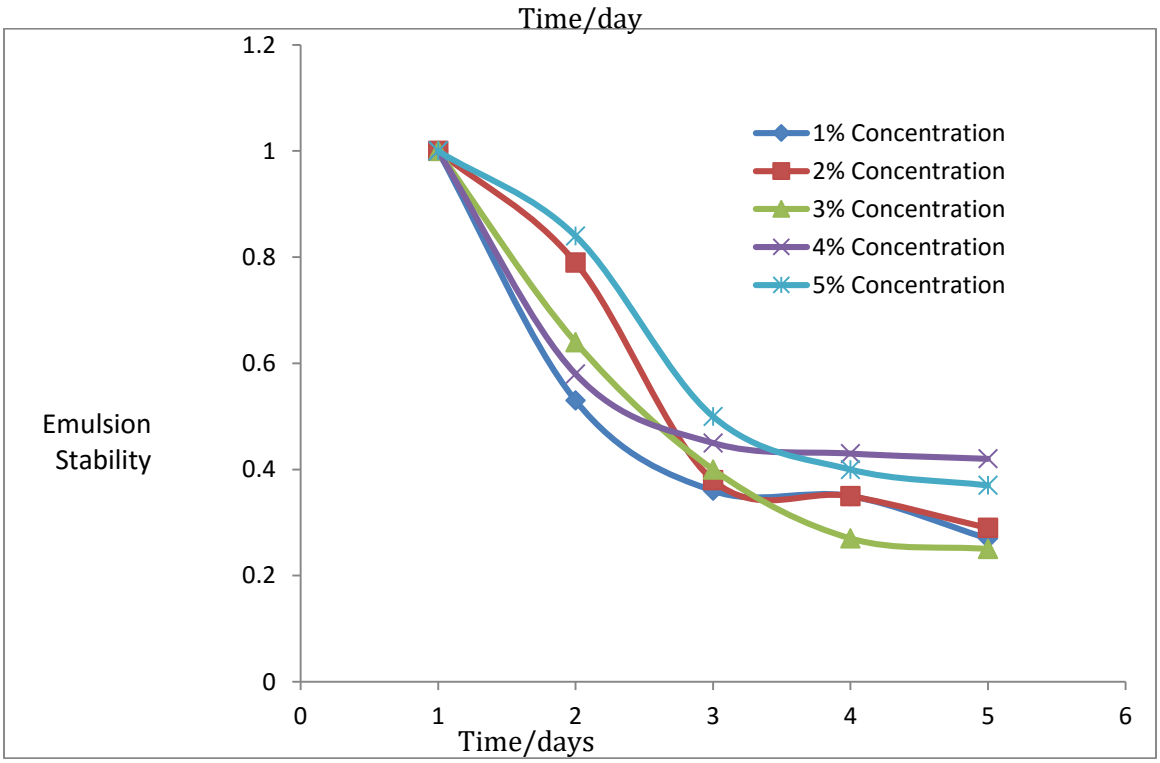

Figure 2: Emulsion Stability of Acacia Senegal at Different Concentrations

As shown in Figure 3, the stability of emulsions with A. Seyal drops 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.

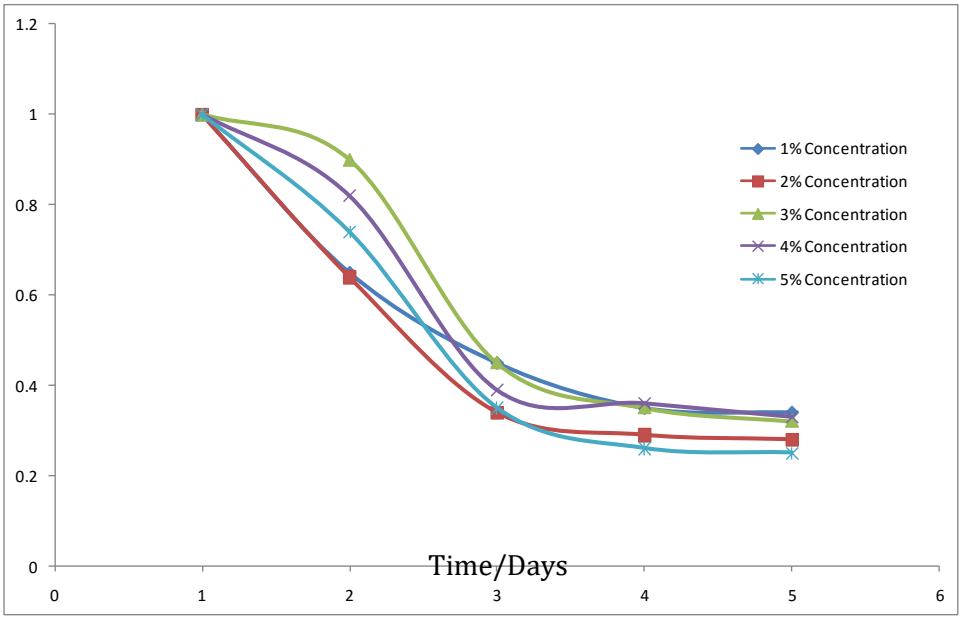

Figure 3: Emulsion Stability of Acacia Seyal at Different Concentration

Figure 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. The stability of emulsion 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 (Akiyama *et al.*, 2005; Song *et al.*, 2000).

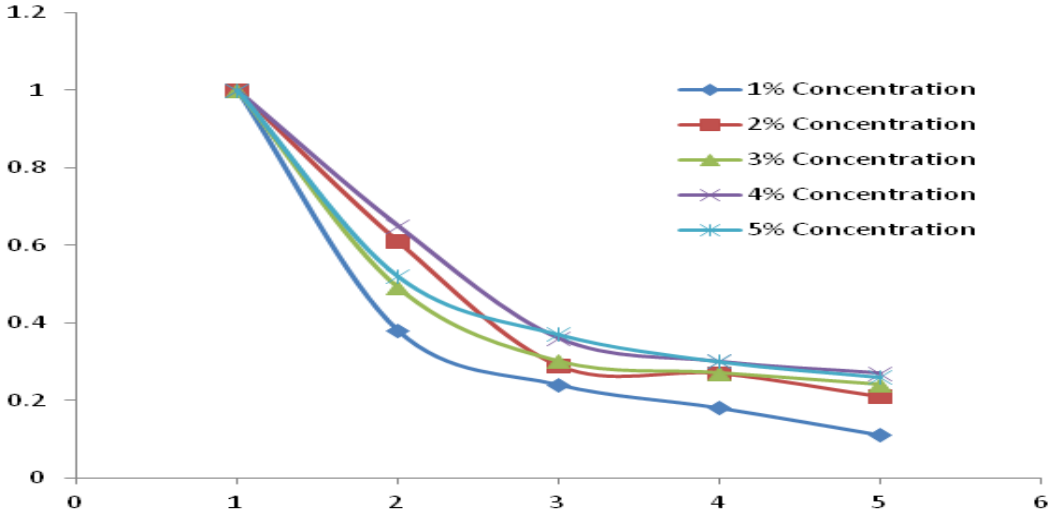

Figure 4: Emulsion Stability of A. Tortilis at Different % Concentrations

Figure 5 shows the stability of emulsion containing Acacia Mellifera at different gum concentration. It is found that the emulsion produce by high gum concentration is more stable than that of emulsion produced by low gum concentration, instability exhibit from first day. Same as the results given by A. Tortilis, it is shown that stability drops sharply within the first 2 days for the lowest concentration (1%), after which the stability values approached a plateau, while for the other concentrations (2%, 3%, 4%, and 5%), the emulsion stability drops sharply within the first 3 days after which the stability values approached a plateau.

These results suggest that varying the concentration of Acacia gum was the most influential variable on ES, and, in general, increasing concentrations of acacia gum resulted in an increase in ES, consequently enhancing its resistance to gravitational separation. Nevertheless, Figures 5 indicate that at any gum concentrations, since the ratio of gum to oil was 2:1, ES range was 80-100% in the first day, however, after the first day the ES drop slowly up to the third day to reach a plateau, where ES decreased to 30-50%. Infact, *Acacia mellifera* gum's stabilizing effect may attributed to its ability to increase the viscosity of the continuous phase, thereby minimizing droplet mobility and decreasing droplet collision numbers (Ye *et al*., 2004).

In this study, it may be suggested that a critical concentration of acacia gums was present, at which a destabilizing effect from acacia gums was prevalent, once surpassed, the apparent viscosity of the continuous phase was increased, thereby reducing the mobility of the emulsion droplets inhibiting aggregation or coalescence (Sworn, 2000).

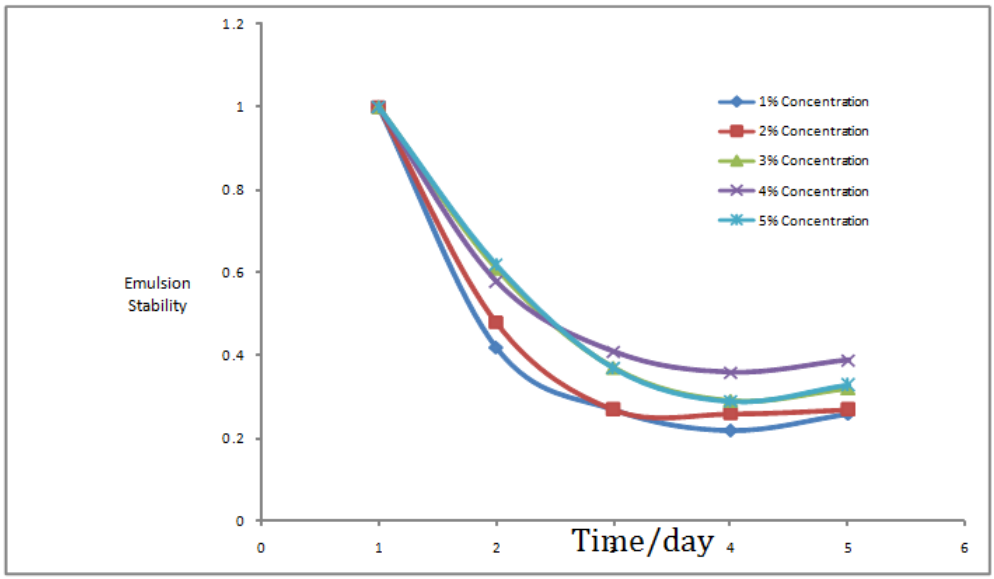

Figure 5: Emulsion Stability of *Acacia mellifera*- Different Concentration

Emulsion Stability

Effect of Protein Concentration

The effect of protein concentration of different acacia gums on the emulsion stability is presented in Figures (6, 7 and 8). As shown, 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.

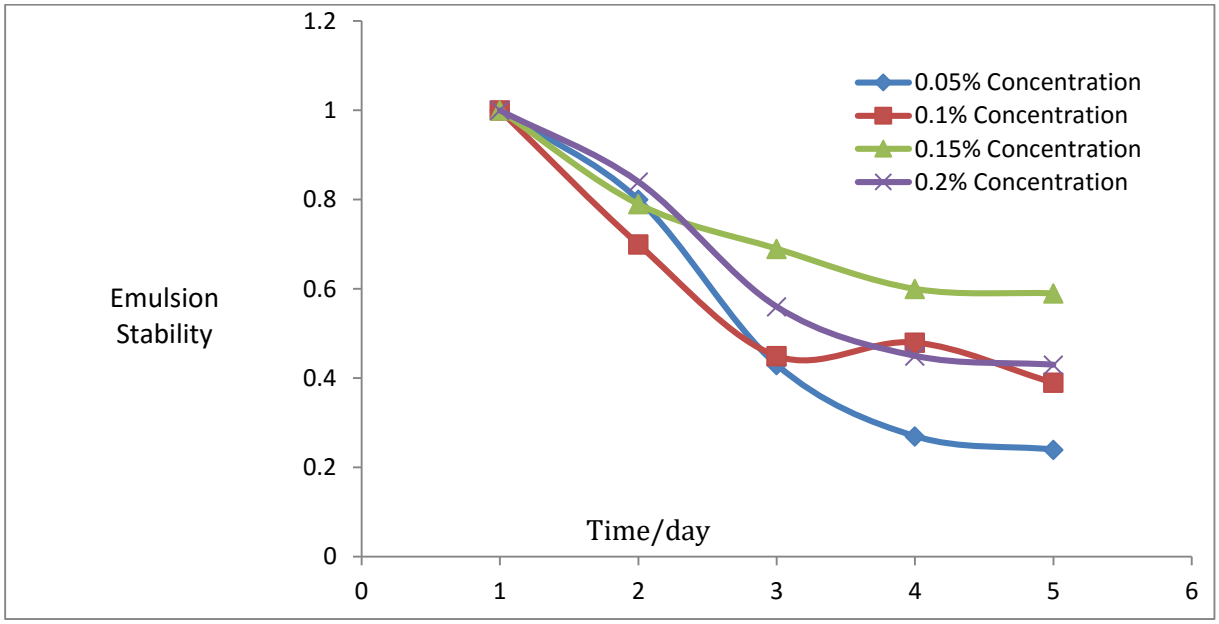

Figure 6: Emulsion Stability of Acacia Senegal- Different Protein Concentration

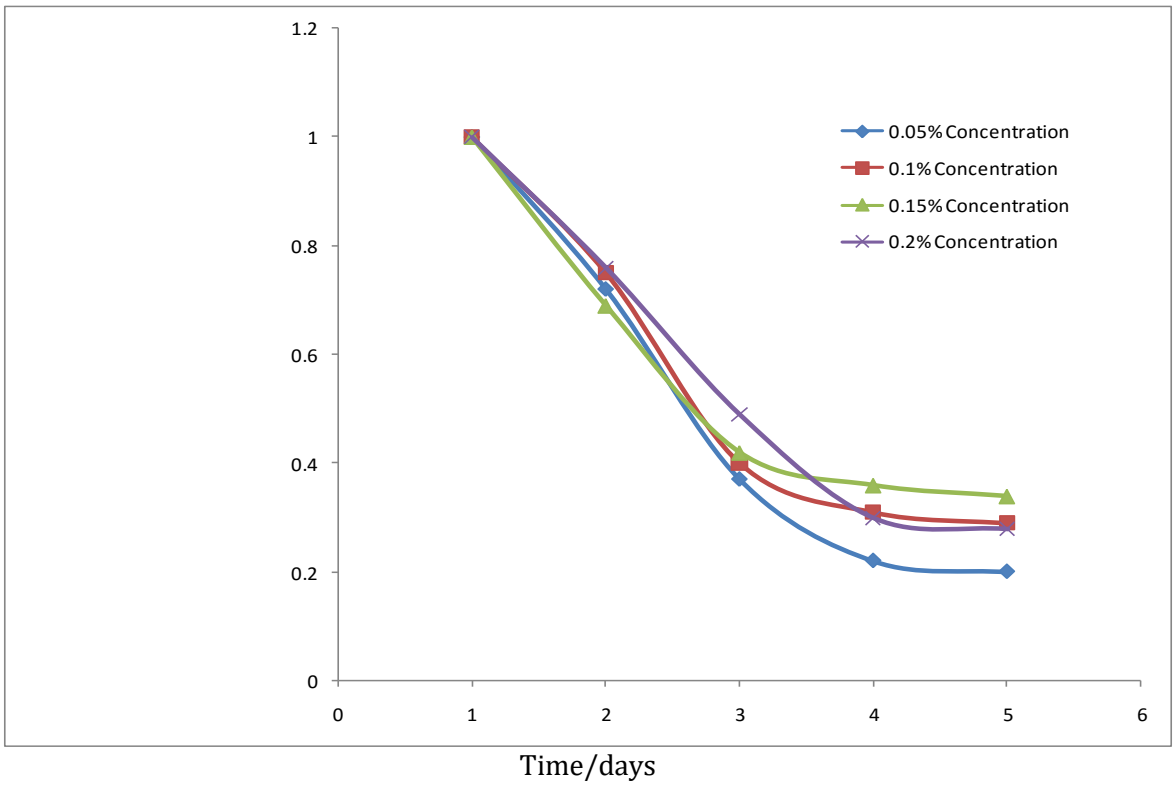

Figure 7: Emulsion Stability of *Acacia seyal* -Different Protein Concentration

Mean Droplet Diameter

Mean droplet size (d) profile of all the emulsions studied is 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 9 all of the curves are monomodal. *A. tortilis* has an extent distribution of particle size from 0.09 to 5 µm and (d) 0.5µm, A. Mellifera 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) 0.6µm, *A. senegal* has an extent distribution of particle size from 0.04 to 1.25 µm and d 0.6µm.

The first observation from figure 9 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 µm at a limited range of size distribution (0.04 to 1.5 µm) which refers to the emulsifying/stabilizing ability of these gums.

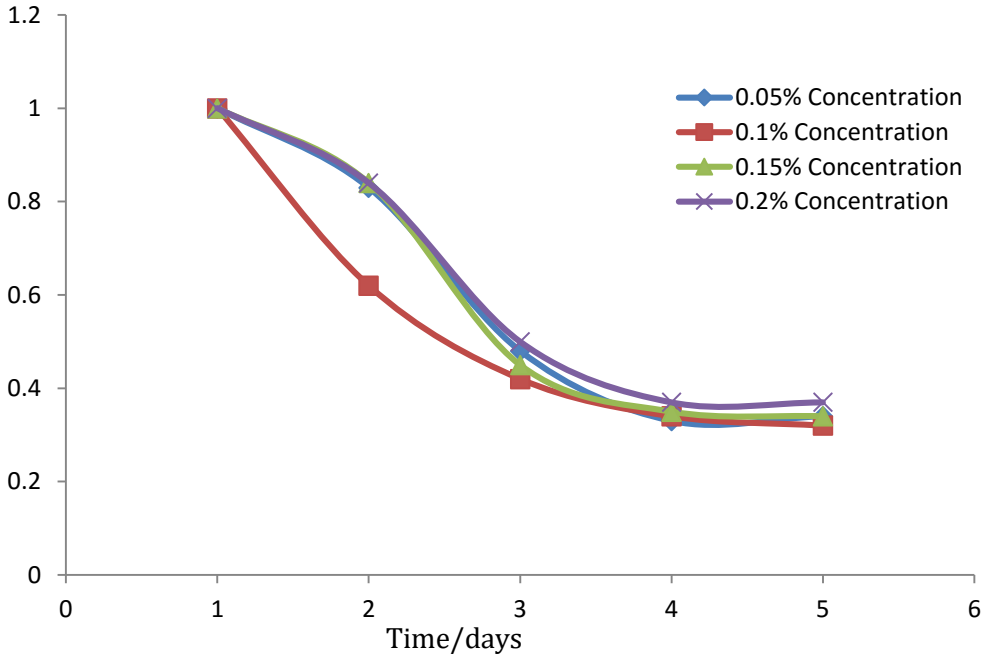

Figure 8: Emulsion Stability of *Acacia tortilis* at Different Protein Concentration

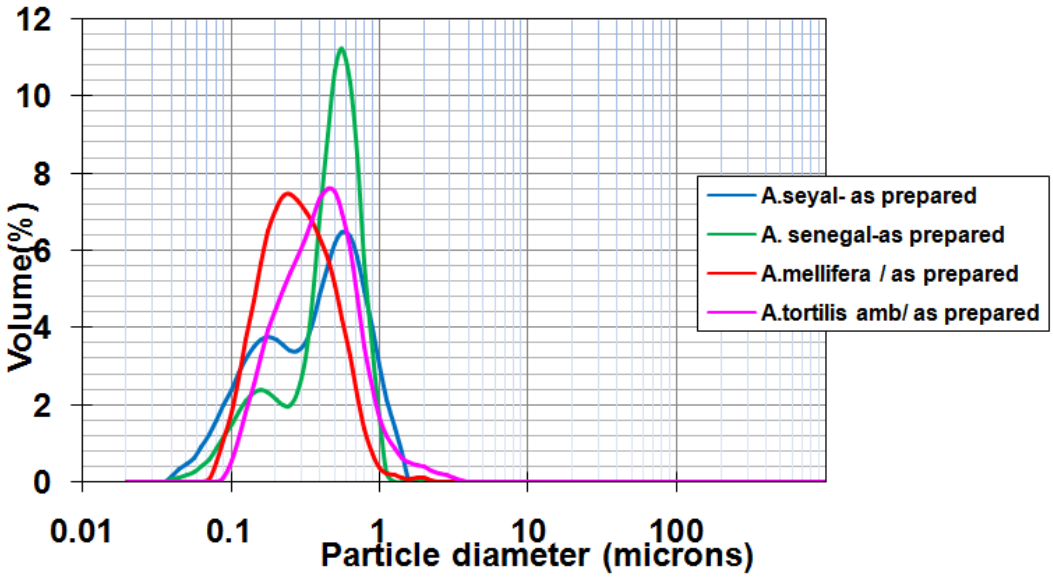

Figure 9: Droplet Size Distribution of D-Lemonine Oil Emulsions with Different Acacia Gums at Zero Time

Emulsion Long-Term Stability Test / Accelerated Temperature Stress Test

In this experiment, the long-term stability of the emulsion for samples of acacia gums were evaluated by using the accelerated stress at 60. The particle size for the stored emulsion at 60 C°was measured by a Mastersizer 2000 'illustrated in figure 10 at given time intervals (3 days and 7 days).

Figure 10 shows droplet size distribution of D-lemonine oil emulsions with different acacia gums after storage for 7 days at 60 ^{0}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 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.

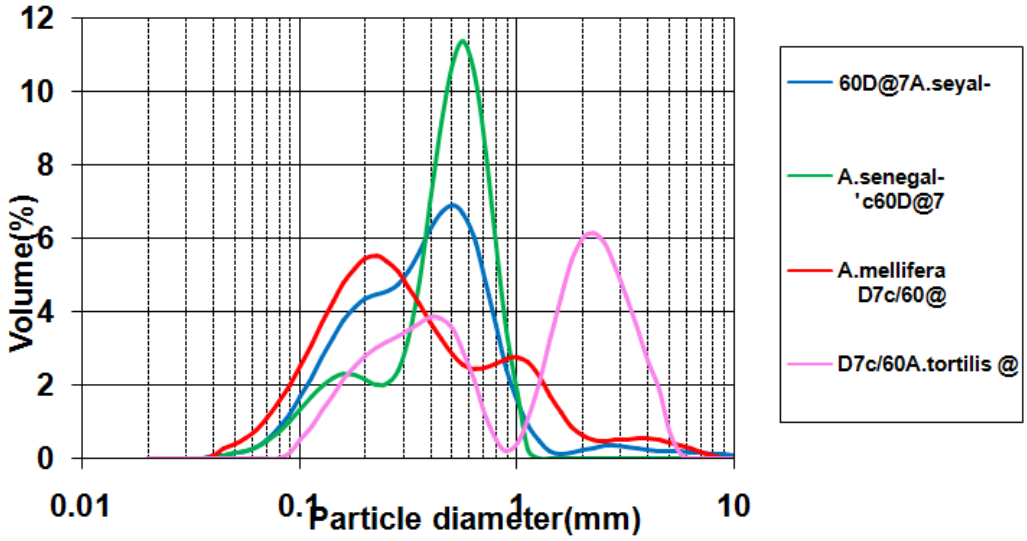

Figure 10: Droplet Size Distribution of D-Lemonine Oil Emulsions with Different Acacia Gums after Storage for 7 Days at 60 ^{0}C

The distribution curve on day 0 is relatively narrow, however, as time increases, the distributions curve becomes wider with a slight shift towards the right, figure 10, indicating that the droplet diameter increased and also that diameter size became less homogenous. The distributions curve becomes wider with a slight shift towards the right indicating that the droplet diameter increased and also that diameter size became less homogenous.

Conclusion

The results indicate that varying the concentration of acacia gum play a significant role in ES, and additions of acacia gum of high protein concentration resulted in an increase in ES of the D-lemonine-in-gum solution emulsion, it is also found that 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 of emulsion stability, contributing greatly to evaluate the physical stability of emulsions.

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