Examination of Stability of Emulsions Containing Two Gums from the *Vulgares* Family and Combinations

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Abstract: Oil - in - water emulsions were prepared using two gums from the Vulgares series, namely Acacia senegal var. senegal (ASG) and Acacia mellifera (AMF), along with a 1: 1 blend of these gums. Various concentrations of Isopropyl Myristate (IPM) oil were utilized, and the emulsions were monitored for four weeks at 45°C. The morphology of the emulsions revealed dense, spherical droplets. Analysis of particle sizes indicated that AMF gum produced smaller and more resilient droplets compared to ASG. Phase separation was observed at lower oil concentrations for both gum - based emulsions. However, the emulsion containing the blended Vulgares gums (in a 1: 1 ratio), prepared with an optimal oil concentration of 20%, exhibited improved stability.

Keywords: Vulgares, Acacia senegal var. senegal, Acacia mellifera, emulsion, stability

1. Introduction

The term emulsion is derived from the word emulgeo meaning "to milk". Milk is example of a natural emulsion. "An emulsion is a heterogeneous system consisting of at least one immiscible liquid intimately dispersed in another in the form of droplets, whose diameter, in general, exceeds 0.1 µm. Such systems possess a minimal stability, which may be accentuated by such additives as surface - active agents, finely divided solids, etc. "Friberg, Mandell [1] elucidated the significance of liquid crystalline phases on emulsion stability. This was reflected in the IUPAC [2] definition of an emulsion: "in an emulsion, liquid droplets and/or liquid crystals are dispersed in a liquid. " Sharma and Shah [3] defined both micro - and macroemulsions, differentiating them on the basis of size and stability. Emulsifiers are added to increase product stability and attain an acceptable shelf life. The function of an emulsifier is to join together oily and aqueous phases of an emulsion in a homogeneous and stable preparation [4]. Food emulsifiers have a wide range of functions. The most obvious is to assist stabilization and formation of emulsions by the reduction of surface tension at the oil-water interface, to alter the functional properties of other food components and third function is to modify the crystallization of fat.

One of the key functional roles of food hydrocolloids is in the preparation of emulsions and in the control of emulsion shelf - life. Product applications include carbonated soft drinks [5], ice - cream [6], and sauces and dressings [7]. Most hydrocolloids can act as stabilizers (stabilizing agents) of oil - in - water emulsions, but only a few can act as emulsifiers (emulsifying agents). The latter functionality requires substantial surface activity at the oil–water interface, and hence the ability to facilitate the formation and stabilization of fine droplets during and after emulsification [8, 9]. The most, widely, used polysaccharide emulsifiers in food applications are gum arabic (Acacia senegal var. senegal), modified starches, modified celluloses, pectin, and some Galacto - mannans [8, 10]. The surface activity of these hydrocolloids has its molecular origin in either (i) the non - polar character of chemical groups attached to the hydrophilic polysaccharide backbone (in hydrophobically modified starch/cellulose) or (ii) the presence of a protein component linked covalently or physically to the polysaccharide (some gums, pectins, etc.). According to Bentham's taxonomic classification of the Acacia genus, Acacia seyal var. seyal (ASY) belongs to series 4 (Gummiferae) and Acacia senegal var. senegal (ASG) to series 5 (Vulgares). There are important differences between the two species which are: ASY gum is less acidic, has positive optical rotation, low viscosity, low rhamnose and arabinose/galactose ratio > 1, on the other hand ASG gum is more acidic, has negative optical rotation, higher viscosity, higher proportion of rhamnose and arabinose/galactose ratio [11, 12]. These differences reflect the differences <1 between Gummiferae and Vulgares series [13]. Acacia gums are widely used to form beverage emulsions, a class of oil - in - water (O/W) emulsions which are unique in that they are consumed in a, highly, diluted form. They are prepared as an emulsion concentrate, which is later diluted several hundred times in acidic sugar solution [14]. Emulsions should be stable to significant degree in both concentrate and dilute form [15]. Thus, stability of beverage emulsions is a chronic problem that plagues the flavor and beverage industries.

The objectives of this work are to prepare, monitor, and evaluate stability of emulsions formed using gum exudate of

Acacia senegal var. senegal (ASG) and Acacia mellifera (AMF) from Vulgares series and their blend.

2. Materials and methods

2.1. Materials

Acacia senegal var. senegal (ASG) gum was provided by Dr. M. E. Osman, Natural Gums Research Centre of Sudan University of Science and Technology (season 2013). Acacia mellifera (AMF) was provided by Gum Arabic Research Center, El Obied, Sudan. Iso Propyl Myristate (IPM) oil was purchased from Spectrum Chemical MFG. CORP., used as the dispersed phase. Deionized water (18.2M Ω cm⁻¹resistivity) was used throughout this work.

2.2. Samples preparations

Gum nodules were dried at room temperature, cleaned by hand, ground using mortar and pestle, and kept in labeled plastic containers for analysis.

2.3. Preparation of emulsions

An appropriate amount of gum samples (based on dry weight) was dissolved in a measured amount of deionized water to make 30% (w/w) gum solutions, samples were dissolved using magnetic stirrer for 3 hours and left overnight for full hydration then centrifuged to remove air bubbles and insoluble particles for10 minutes at 2500 rpm and 25°C [16]. The solution was filtered using 100 µm mesh. Varying amounts of IPM oil were added to an appropriate amount of aqueous gum solution to make (20% -10% w/w) oil concentration and (20% w/w) gum concentration. Emulsions were prepared using (ULTRA TURRAX T25 basic IKA) homogenizer at 24, 000 rpm for 5 minutes. Emulsions formed were kept in incubator of 45.0±0.1°C subjected to accelerating aging process. Stability of emulsions was determined from observing emulsion's physical changes over four weeks. Blend Emulsion was prepared using combination of the two Vulgares gums with IPM oil, to achieve 20% total gum content and 20% oil for a total of 10 g. Gums of ASG and AMF were mixed 1: 1 (w/w).

2.4. Morphology of Emulsion

Light polarizing microscope from Leica model PM RXP by Leica Microsystems GmbH, Germany was used to observe the emulsion droplets formed. Polarizing microscope unit was equipped with high voltage beam, polarizing unit and a JVC Color Video camera with model KY F550, interfaced with personal computer with Leica QWin image analysis software. Leica QWin software is a powerful tool in digital images capturing with capability of measuring and analysis of droplet size of sample. Samples were examined under the microscope with previous dilution in deionized water (three times w/w). A drop of emulsion was deposited on a microscope slide and then covered with a cover slip [17]. Observation of changes in droplets size and appearance of emulsion of the *Acacia* gums under the study has been made for one and seven days of incubation at $45^{\circ}C$.

2.5. Particle Size Analysis

The average particle size of *Acacia* gum emulsions was determined using (COULTER N4 PLUS), 0.05g of emulsions were diluted with 5ml deionized water. A 1cm path length clear quartz cuvette was used for particle size measurements at 25° C.

2.6. Phase Separation Stability Test

Destabilization of *Acacia* gums and blends emulsions in terms of phase separation was observed by measuring heights of phases and calculating emulsion fraction as percentage [18, 19], while incubating the emulsions for four weeks at 45° C.

3. Results and discussions

3.1. Emulsion Morphology

The morphological structures of the two *Vulgares* gum species emulsions were studied over a period of seven days of incubation at 45° C and different IPM oil concentrations. The oil droplets were spherical in shape and retain their structure in spite of dense emulsion reflecting a high degree of stability and elasticity of droplets exhibited by each gum. *Acacia* gums increase the viscosity of the continuous phase and form a network around the droplets. Figures (1 & 2) show the morphology of ASG and AMF gum emulsions; AMF gum formed smaller droplets than ASG gum. Figure (3); shows the morphology of the emulsion formed by a blend of ASG and AMF gums (1: 1); and it was observed that the droplets formed were smaller than that formed by the parent gums.

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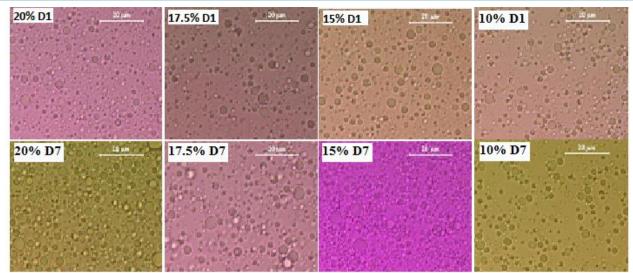


Figure 1: Micrographs of ASG gum emulsion samples with different IPM oil concentrations; 20%, 17.5%, 15%, and 10%; after one day (D1) and seven days (D7) of incubation at 45°C.

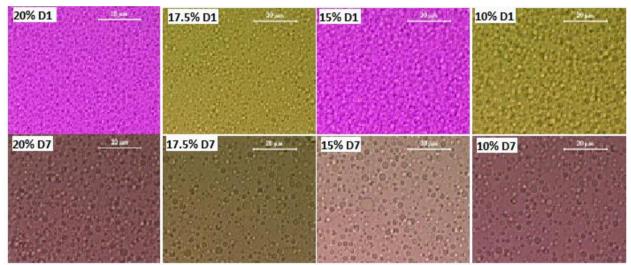


Figure 2: Micrographs of AMF gum emulsion samples with different IPM oil concentrations; 20%, 17.5%, 15%, and 10%; after one day (D1) and seven days (D7) of incubation at 45°C.

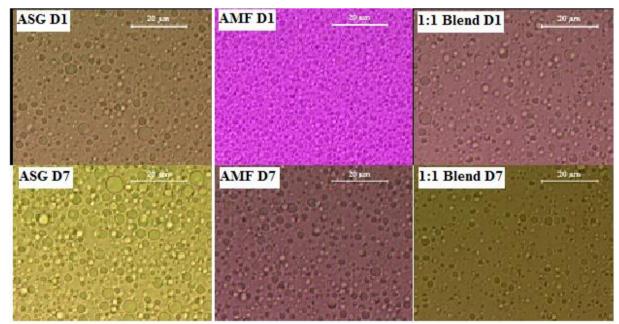


Figure 3: Micrographs of ASG, AMF, and 1: 1 gum blend emulsion samples with 20% IPM oil concentrations after one day (D1) and seven days (D7) of incubation at 45°C.

3.2. Particle size analysis

Vulgares gum emulsions were analyzed with COULTER N4 PLUS to measure droplet size. The average droplets size was an average of three measurements. Data of droplets size measurements were tabulated (Tables 1&2). Figures (4&5) show the average droplets size for each gum emulsion using different IPM oil concentrations and that ASG gum emulsions exhibit the larger average droplet size than AMF gum emulsions. Figure (6) shows the average droplets size of the two *Vulgares* gums and their 1: 1 blend emulsion. It was found that the smallest average droplets size formed by the blend.

Table 1: Average drop	lets size of ASG gu	m emulsions
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Number	Average Droplets Size (nm)				
of Days	20% IPM	17.5% IPM	15% IPM	10% IPM	
Day1	2397.1	2974.3	2997.9	2798.1	
Day3	2425.4	2835.3	2799.9	2711.2	
Day7	2952.4	2814.6	2819.5	2960.4	
Day14	3020.6	2403.2	2666.3	3086.7	
Day21	3078.6	2867.2	2970	2996.7	
Day28	3071.1	3119.2	3313.3	3132.5	

Table 2: Average droplets size of AMF gum emulsions

Number	Average Droplets Size (nm)				
of Days	20% IPM	17.5% IPM	15% IPM	10% IPM	
Day1	2524.0	2314.6	2738.4	2581.8	
Day3	2586.3	2874.9	3051.8	2626.3	
Day7	2790.5	2800.9	3213.6	2739.6	
Day14	2802.5	2928.5	3375.2	2987.6	
Day21	2814.5	2986.2	3097.3	3076.1	
Day28	2841.8	2996.5	3190.6	3096.9	

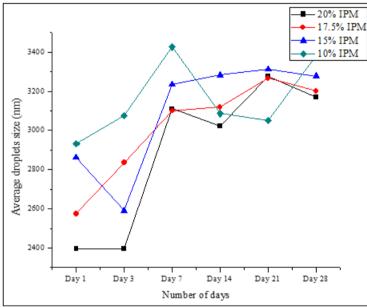


Figure 4: Variation of average droplet size of ASG gum emulsion with time at various IPM concentrations incubated at 45°C.

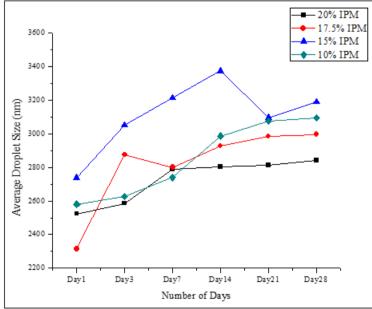


Figure 5: Variation of average droplet size of AMF gum emulsion with time at various IPM concentrations incubated at 45°C.

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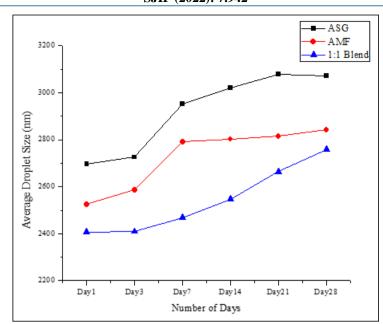


Figure 6: Variation of average droplet size of ASG, AMF and their 1: 1 gums blend emulsions with time at 20% IPM oil concentration incubated at 45°C for 28 days.

3.3. Phase Separation Stability Test

Emulsion fractions were measured for *Vulgares* gums emulsions and their blend incubated at 45° C over 28 days (Figures 7 - 9). It was observed that AMF gum emulsion was more stable against phase separation than ASG gum emulsion. These findings were supported by the previous results and the 20% oil concentration emulsion was the best concentration for both gums as emulsions with lower oil concentrations were less stable. Gums blend emulsion was prepared with 20% IPM oil concentration as it was the concentration of the best emulsion stability. Figure (9) shows the emulsion fraction percentage for ASG, AMF, and 1: 1 blend proportion; which was more stable against phase separation than the emulsions of parent gums.

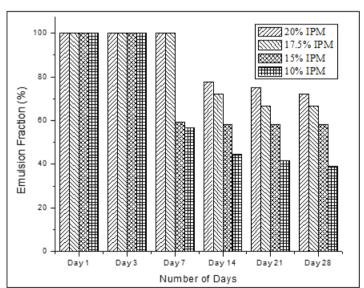


Figure 7: ASG Gum Emulsion Fraction% at various IPM oil concentrations incubated at 45°C for 28 days.

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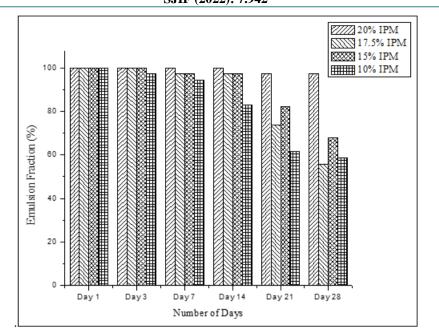


Figure 8: AMF Gum Emulsion Fraction% at various IPM oil concentrations incubated at 45°C for 28 days

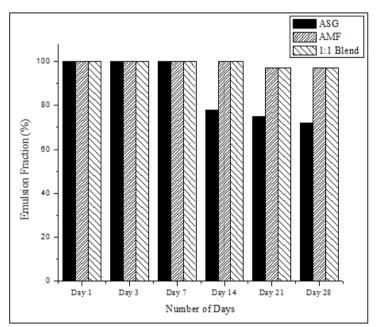


Figure 9: Emulsion fraction% of ASG, AMF, and 1: 1 gum blend with 20% IPM oil concentration incubated at 45°C for 28 days

4. Conclusions

Emulsions formed with ASG and AMF gums are remarkably stable and that the stability increases as the oil concentration increases. It was also found that the AMF gum produces smaller droplets, and more stable emulsions than ASG gum that ASG gum emulsions were more sensitive to the factors affecting emulsion stability. Blended gums emulsion showed stability that was better than the emulsions formed with parent gums reflecting the viability of blending to enhance emulsion stability.

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