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FULL LENGTH ARTICLE

Characterization and functional properties of some natural *Acacia* gums

Rabeea M.A. Daoub [a,b,*], Aarif H. Elmubarak [c], Misni Misran [b], Elfatih A. Hassan [a], Mohammed E. Osman [a]

[a] *Sudan University of Science and Technology, P.O. Box 407, Khartoum, Sudan*
[b] *Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[c] *Department of Plant Protection, College of Food & Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia*

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Abstract Authentic representative gum exudate samples from *Acacia* species namely *Acacia senegal* var. *senegal* (ASG), *Acacia mellifera* (AMF), *Acacia seyal* var. *seyal* (ASY), and *Acacia tortilis* var. *raddiana* (ATR), were physicochemically analyzed. The moisture, ash, nitrogen and protein content, pH, specific optical rotation, and number average molecular weight were found to be ranging from 9.76% to 8.35%, 3.40% to 2.05%, 0.243% to 1.549%, 1.610% to 10.378%, 4.45 to 4.94, −48.25 to +86.75 and 0.24×10^6 to 2.95×10^6 respectively. The ^{13}C and ^{1}H NMR spectra of gum samples showed similarity in individual sugar components, but characteristic patterns of each gum, were observed. FTIR spectra of the studied gums show the presence of the same functional groups in the four gums. DSC and TGA thermograms were characteristic for each gum. Evaluation of the functional properties of the four gums indicated that ATR gum bears the best emulsification characteristics in terms of emulsion's stability and emulsification power.

1. Introduction

Gums are natural exudates from trunk, branches or fruit of trees due to scission, injury (whether incidental or deliberate) or fungal infection. They are used in foods (Glicksman, 1982; Salve, 2011; Walker, 1984), pharmaceutical and many other industries (FAO, 1996). Gums are hydrocolloids polysaccharides (Williams and Phillips, 2000). The most utilized gum type is Gum Arabic (GA) obtained from *Acacia senegal var. senegal* trees. The wide use of GA is due to its high solubility and low viscosity compared to other polysaccharides, its good emulsifying characteristics and its non-toxic nature. Other gum types may be used as a substituent of GA after the study of their physicochemical properties and functionalities (Taha et al., 2012).

Gums from different *Acacia* species exhibited, intrinsically, different characteristics. Even gums of different varieties

* Corresponding author at: Sudan University of Science and Technology, P.O. Box 407, Khartoum, Sudan.
E-mail address: rabiea.daoub@gmail.com (R.M.A. Daoub).
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within same species, bear some differences. It is also noted that gums of the same variety grow in different locations produce gums that are characteristically different. Recognition of differences in the species, varieties and environment is important in producing gums for a desired end use (Chikamai, 1998; Yebeyen et al., 2009). *Acacia* gums are unique hydrocolloids in that they are water soluble. They are, notably, used in food industry to control and modify the rheological properties of aqueous food systems. *Acacia* gums act as stabilizers, film formers, thickeners, flocculants, suspending agents and emulsifiers. *Acacia senegal* var. *senegal* gum in choice superior position is compared to *Acacia seyal* var. *seyal* gum although both are considered Gum Arabic in the international market, because it is better emulsifier and stabilizer than *Acacia seyal* var. *seyal* gum (Elmanan et al., 2008), and its solution is, generally, less colorful. These differences in properties explain the higher price of *Acacia senegal* var. *senegal* gum compared to *Acacia seyal* var. *seyal* in the international market (Vanloot et al., 2012).

The identification of a particular gum from a series of different gum exudates needs an extensive number of analytical tests to perform. This approach enables "a chemical finger print" of each gum to be determined. The five most important parameters that can be used to identify raw gums mostly used as food additives are as follows: (1) Specific optical rotation, (2) Nitrogen content, (3) Ash content, (4) Moisture content and (5) Absence of tannins (Karamalla, 1999). The most fundamental properties of a gum which make it unique among polysaccharide, generally, are high water solubility and low viscosity. The majority of gums dissolve in water at different concentrations but Gum Arabic readily dissolves in cold and hot water in concentrations up to 50% W/W (Hassan, 2000; Karamalla, 1999).

Over the years, numerous publications have appeared on the NMR of food polysaccharides, and NMR has become a routine method of analysis (Cheng and Neiss, 2012).

Recently, the technique of Attenuated Total Reflectance (ATR) has revolutionized solid and liquid sample analysis as it eliminates drawbacks of infrared analysis such as sample preparation and spectral reproducibility. The accessory ATR measures the changes that occur in an internally, totally, reflected infrared beam as it comes into contact with the sample.

Differential Scanning Calorimetry (DSC), is a powerful physical technique that monitors physical and chemical changes in the polysaccharide that occurs during thermal processing yielding unique curves for a given polysaccharide (Bothara and Singh, 2012). DSC is most often used because it is fast, simple and readily accessible. DSC instrument involves a sample and a reference taken in holders, and temperature is ramped at a specified rate, or the heater that holds the DSC at a given temperature and the rate of change of heat flow between the sample and the reference is measured, and the corresponding processes that take place in the sample may be inferred from the pattern of the change of heat flow with time during heating the sample from ambient temperature to its decomposition temperature. A simple and accurate technique for studying the decomposition pattern and the thermal stability of polymers is the Thermogravimetric analysis (Bothara and Singh, 2012). The mass of a sample in a controlled atmosphere is monitored, continuously, as a function of time or temperature as the temperature of the sample is increased linearly with time. Thermogram or a thermal decomposition curve was obtained as a plot of mass or mass percentage as a function of time.

The objective of this work was to highlight the differences between some *Acacia* gums samples namely *Acacia senegal* var. *senegal* and *Acacia mellifera* from the *Vulgares* series and *Acacia seyal* var. *seyal* and *Acacia tortilis* var. *raddiana* from the *Gummiferae* series using different physicochemical methods, NMR spectroscopy, FTIR, and thermal analysis: DSC and TGA and to use these gums as emulsifiers and evaluate their functionalities.

2. Materials and methods

2.1. Materials

Acacia senegal var. *senegal* gum (ASG) and *Acacia seyal* var. *seyal* gum (ASY) were provided by Natural Gums Research Centre of Sudan University of Science and Technology (season 2013). *Acacia mellifera* gum (AMF) was obtained from Gum Arabic Research Center, El Obied, Sudan. *Acacia tortilis* var. *raddiana* gum (ATR) was collected by the author (R.M.A. Daoub) from Wd-Mahala Forest 45 km southeast Khartoum, with the assistance of the staff of Forests National Corporation (FNC), Sudan. Iso Propyl Myristate (IPM) oil from Spectrum Chemical MFG. CORP., was used as the dispersed phase. Deionized water was used throughout this work (see Fig. 1).

2.2. Sample 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. Physicochemical properties of gums

Gum samples were analyzed for moisture, ash, nitrogen content, and specific optical rotation following AOAC procedures (AOAC, 1990).

2.4. Nuclear Magnetic Resonance (NMR)

JEOL JNM-ECX500 spectrometer was used to obtain ^{1}H and ^{134}C NMR spectra. Deuterium oxide was used to dissolve *Acacia* gum samples (≈2% w/w) at 90 °C for 3 h before NMR analysis. ^{1}H and ^{13}C NMR spectra were recorded using a standard single-pulse sequence. Measurements were conducted at 50 °C (Nie et al., 2013b).

2.5. ATR–FTIR spectroscopy

A Perkin–Elmer ATR–FTIR Spectrometer Spectrum 400 was used to obtain FTIR spectra. 400–4000 cm^{-1} was the scanning range and 1 cm^{-1} was the resolution. After the crystal area was cleaned, the solid material was placed onto the small crystal area; the pressure arm was positioned over the crystal/sample area. Force was applied to the sample, pushing it onto the diamond surface and the spectrum was collected.

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Figure 1 Gum samples.

2.6. Differential scanning calorimetry (DSC)

DSC Q20 V24.10 Build 122, Module DSC Standard Cell FC, a Pan (T zero Aluminum Hermetic) and Nitrogen gas at flow rate: 50.0 ml/min. Sample of gum species (about 5 mg) was placed in a pre-weighed aluminum sample pan and the pan was sealed using a Quick Press pan crimper and the thermal data between 30 °C and 400 °C in nitrogen atmosphere with a heating rate of 10 °C min^{-1} were recorded. An empty pan served as the reference.

2.7. Thermogravimetric Analysis (TGA)

Netzsch, TG 209 F3 Tarsus instrument that runs under Proteus® Software on Windows® operating system was used. About 13 mg of gum samples was weighed in sample pans and the weight loss against temperature from ambient to 400 °C at a heating rate of 10 °C min^{-1} was recorded.

2.8. 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, and samples were dissolved using magnetic stirrer for 3 h and left overnight for full hydration and then centrifuged to remove air bubbles and insoluble particles for 10 min at 2500 rpm and 25 °C (Sanchez et al., 2002). The solution was filtered using 100 μm mesh. Varying amounts of Isopropyl myristate (IPM) oil were added to an appropriate amount of aqueous gum solution to make (10–20% w/w) oil concentration and (20% w/w) gum concentration. Emulsions were prepared using Ultra Turrax T25 basic IKA homogenizer at 24,000 rpm speed for 5 min. Emulsions formed were kept in incubator at 45.0 ± 0.1 °C subjected to accelerating aging process. Stability of emulsions was determined from observing emulsion's physical changes over four weeks.

2.9. Particle size analysis

The average particle size of *Acacia* gum emulsions was determined using Coulter N4 Plus instrument, where 0.05 g of each emulsion was diluted with 5 ml deionized water. A 1 cm path length clear quartz cuvette was used for particle size measurements at 25 °C.

2.10. Flow rheological measurements

For rheological measurements CVO-R Rheometer Germini™, Malvern Instrument UK with Peltier Plate temperature regulator was used. The emulsion sample was placed in 4°/40 mm cone and plate geometry with solvent trap at 25 °C. Flow profile was measured for emulsions of the *Acacia* gum species ASG, AMF, ASY and ATR. Measurements were done after one day incubation at 45 °C of the emulsions.

3. Results and discussion

3.1. Physicochemical properties of gums

Table 1 shows analytical data of the samples under the study. Analysis of samples was carried out in triplicate and then averaged.

Table 1 Physicochemical properties of the gum samples.

Gum	Moisture (%)	Ash (%)	Nitrogen (%)	Protein (%)	pH	Specific optical rotation	$M_n \times 10^6$
ASG	9.76	3.40	0.327	2.158[a]	4.94	−31.75	0.24
AMF	9.56	2.50	0.630	4.158[a]	4.53	−48.25	2.01
ASY	8.35	3.13	0.243	1.610[b]	4.84	+56.00	2.95
ATR	8.49	2.05	1.549	10.378[c]	4.45	+86.75	2.06

[a] 6.6 NCF (Anderson, 1986).
[b] 6.625 NCF (Osman, 1993).
[c] 6.7 NCF (Abdelrahman, 2011).

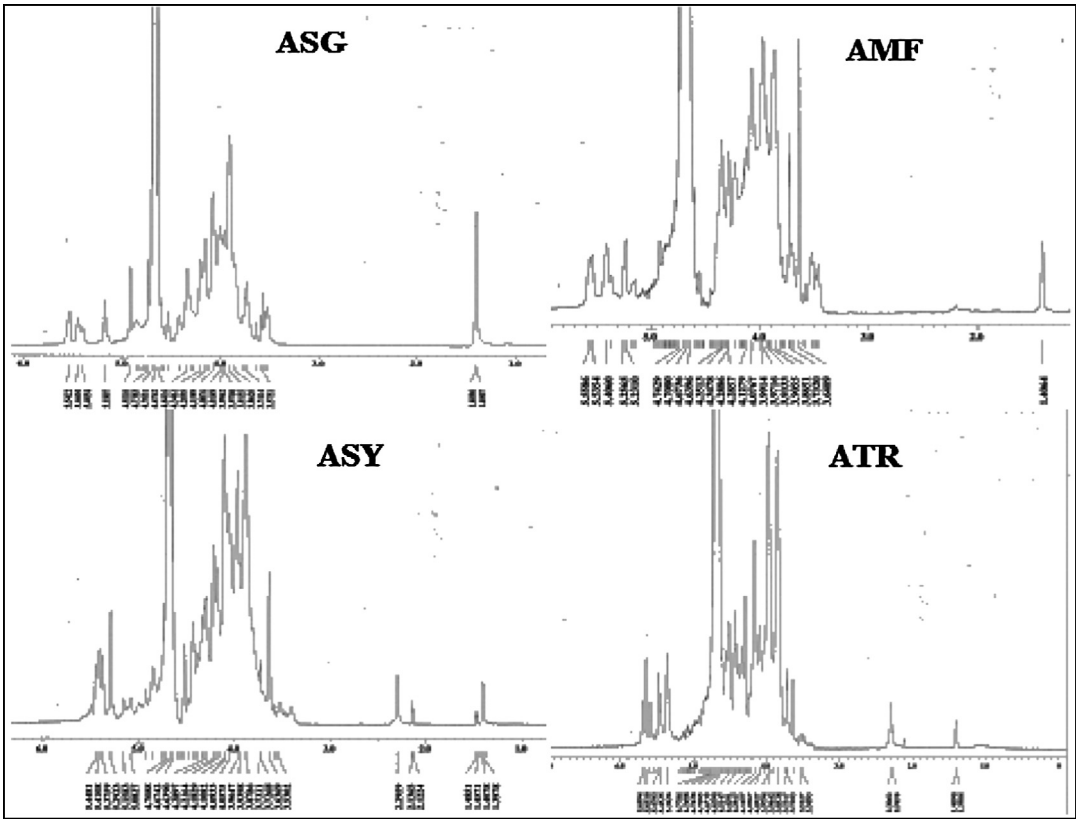

Figure 2 ^{1}H NMR spectra of ASG, AMF, ASY, and ATR gums.

The physicochemical properties for the *Acacia* species gums fall within the range of the specifications of the gums reported by Karamalla et al. (1998), Al-Assaf et al. (2005), Hassan et al. (2005), Elmanan et al. (2008), and Abdelrahman (2011).

While gums from *Vulgares* series (ASG and AMF) showed negative optical rotation, *Gummiferae* series gums (ASY and ATR) showed positive optical rotation of the polarized light. The nitrogen and consequently the protein contents of the four gums were different even within the same series. This was seen from the high protein content of the ATR gum from the *Gummiferae* series and the very low protein content of ASY gum from the same series (Churms et al., 1986). The number average molecular weight was higher for the *Gummiferae* gums than for *Vulgares* gums. The pH values for the gums were in the range from 4.45 to 4.94, showing that all gum samples were slightly acidic due to the presence of free carboxyl groups of D-glucuronic acid and 4-O-methyl D-glucuronic acid residues (Karamalla et al., 1998).

3.2. NMR spectroscopy

Figs. 2 and 3 show ^{1}H and ^{13}C NMR spectra of *Acacia* polysaccharide gums (ASG, AMF, ASY, and ATR). Typical NMR spectra were reported for ASG (Nie et al., 2013b) and for ASY (Nie et al., 2013a).

Fig. 2 shows crowded signals in the ^{1}H NMR spectrum between 3 and 6 ppm which is typical of polysaccharides and reflects the presence of similar sugar residues, for each gum an upfield peak at around 1.4 ppm assigned to the methyl group of rhamnose sugar which on high resolution shows triplet of triplets. The chemical shift at 2.13 ppm in the ASY

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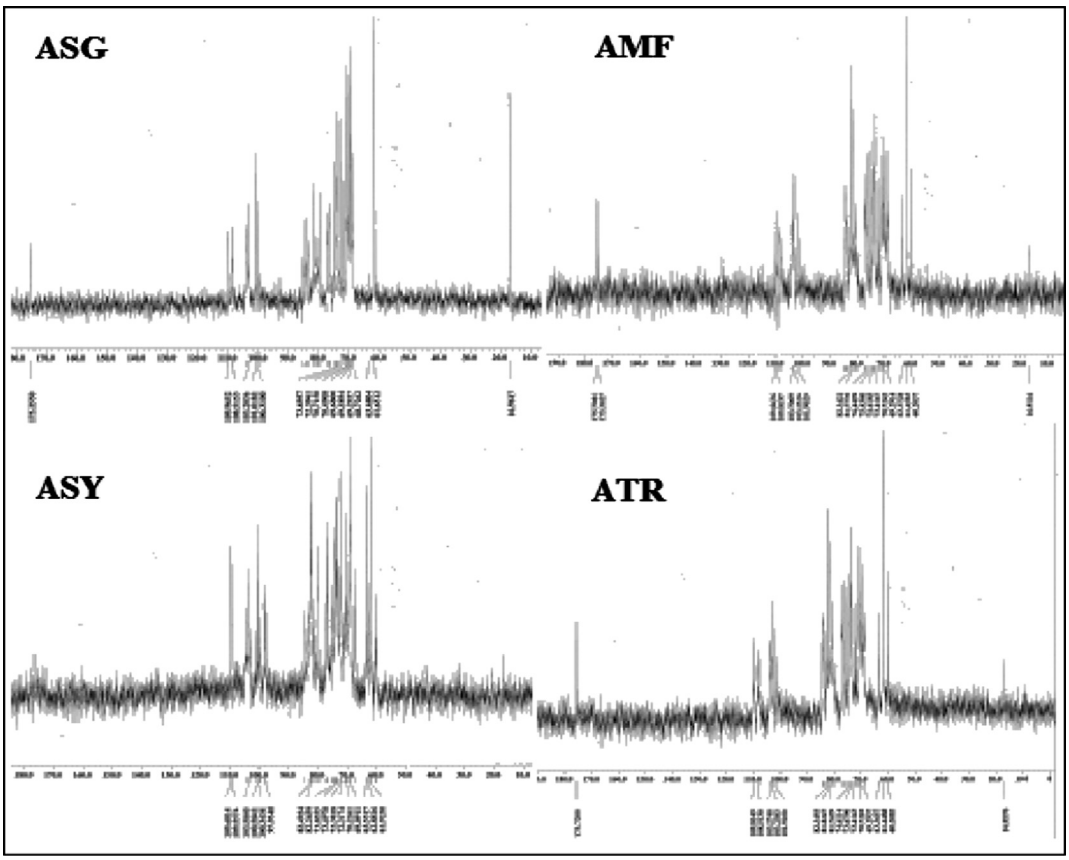

Figure 3 ^{13}C NMR spectra of ASG, AMF, ASY and ATR gums.

and ATR gums spectra indicates the presence of an acetyl group ($COCH_3$). Signals arise at 3.3–3.8 ppm were due to the presence of —O—CH_3. The non-anomeric protons (H_2–H_6) were assigned between 3.3 and 4.6 ppm. The high intensity peak at 4.6–4.80 ppm is partially due to the presence of H_2O. In the anomeric region (4.8–5.8 ppm), more than ten peaks were observed, clearly, in the ^1H NMR spectra of the gums (Nep and Conway, 2010).

From ^{13}C spectra of ASG, AMF, ASY, and ATR gums (Fig. 3), the peaks at 16.96, 16.91, 16.21, and 16.95 ppm respectively, belong to the carbon of methyl group of rhamnose, and this indicates that gums contain deoxygenated sugars (Cui, 2005). While the signals due to non-anomeric carbons C_2–C_5 appear between 60 and 85 ppm (Nep and Conway, 2010), signals from anomeric carbons of the monosaccharide components appear in the 90–110 ppm (Cui, 2005), and for each gum the peak at around 175 ppm arises from uronic acid typical of C_6 signal.

3.3. ATR–FTIR spectroscopy

In the FTIR spectra of ASG, AMF, ASY, and ATR gums, Fig. 4, a characteristic absorption band at 3290–3305 cm^{-1} representing the presence of hydrogen bonded OH group was observed. The characteristic absorption band in the region of 3400–3500 cm^{-1} for amino group must have been masked by the broad O—H group absorption band. The bands at 2926 cm^{-1} indicate the presence of sugars, galactose, arabinose, and rhamnose, also the presence of alkane C—H stretch and aldehyde C—H stretch. The polymers also showed the characteristic band of C═C stretch, amide NH bend, NO_2 from both aliphatic and aromatic galactoproteins, and amino acids around 1602 cm^{-1}. The glucuronic acids have specific vibrations such as the band at 1411 and 1363 cm^{-1} due to C═O symmetric stretching and —OH bending, respectively. Alkane CH_3 bend, Aromatic C═C stretch, ketone C—C stretch, carboxylic acid C—O stretch, Anhydrides C—O stretch, Amine C—N stretch from Polysaccharides and Galactoproteins were observed at 1377 cm^{-1} band. The band at 1264 cm^{-1} represents alkane CH_3 bend, alcohol C—O stretch, ether C—O—C stretch, carboxylic acid CO stretch, amines C—N stretch, and alkyl due to sugar backbone showing alkane bend, alcohol stretch. Ether stretch is due to attachment of two galactose sugars, and CO and CN stretches from galactoproteins. A distinct band at around 1029 cm^{-1} represents alkene C—H bend from polysaccharides for all gum samples.

3.4. Differential scanning calorimetry (DSC)

Fig. 5 shows the DSC thermograms of ASG, AMF, ASY, and ATR gums. It is observed that endothermic peaks appear at temperatures ranging from 100 °C to 150 °C and exothermic peaks appear at temperatures from 300 °C to 315 °C. The endothermic peaks are due to the loss of water content in these gums. The exothermic peaks correspond to the decomposition of the gums. The thermogram of ASG gum showed a broad endothermic peak which might be associated with the loss of crystallization water, hydrogen bounded water and other

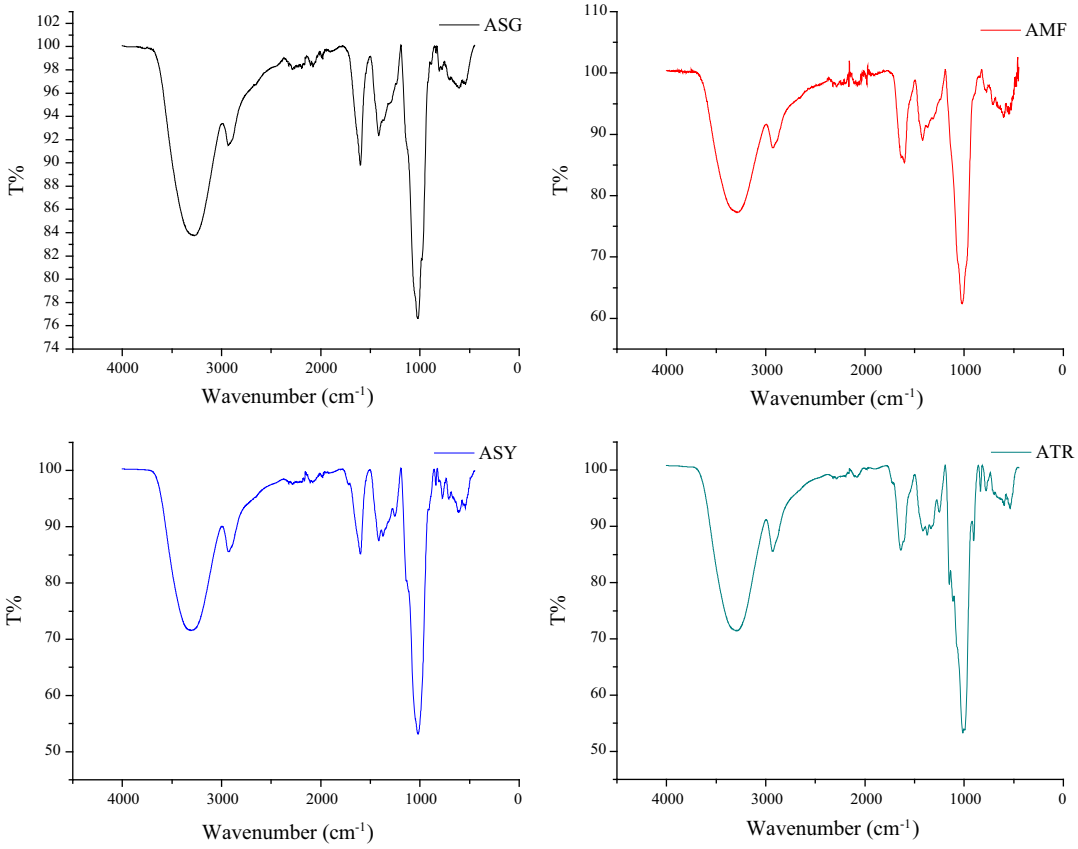

Figure 4 ATR–FTIR spectra of ASG, AMF, ASY, and ATR gums.

physically adsorbed water. For AMF gum thermogram the endothermic peak was sharp which was associated with the loss of water of crystallization indicating a more crystal uniformity than ASG gum which exhibited broad endothermic peak which might be attributed to a less regular packing for ASG, and the AMF exothermic peak reflects small energy flow accompanying the decomposition of the gum.

3.5. Thermogravimetric Analysis (TGA)

Fig. 6 shows representative plots result from thermogravimetric analysis carried out on the ASG, AMF, ASY, and ATR gum samples under nitrogen atmosphere. The thermal analysis results are summarized in Table 2.

The primary thermograms show the thermal stability data and thermal behavior for the gum samples. Heating of the gum samples from 30 to 400 °C, at 10 °C per minute rate, results in two mass loss events. The first mass loss is due to the loss of adsorbed and structural water of gums that take place between 30 and 150 °C as described by other authors (Nep and Conway, 2010), or is attributed to desorption of moisture as hydrogen-bounded water to the polysaccharide structure. The polysaccharide decomposition is related to the second mass loss with an onset temperature of about 260 °C resulted in a 60% mass loss (Zohuriaan and Shokrolahi, 2004). Mass residues of the different gums were ranging from 30% to 35%. The decomposition onset temperature of 260 °C suggests that polysaccharide gums are thermally stable.

The onset temperatures associated with the second mass loss were characteristic for the different gum samples implying different gum structures and different degrees of stability.

3.6. Particle size analysis

Fig. 7 shows the average droplet size of the different *Acacia* gums emulsions at 20% IPM oil concentration. All emulsions were subjected to accelerated test on incubation at 45 °C.

From Fig. 7 ASG gum emulsion exhibits the largest average droplet size and ATR gum emulsion shows the smallest average droplet size. In terms of increasing droplet size the four gums can be arranged in the following order ATR < ASY < AMF < ASG. Some authors attribute the emulsifying power of gums to the protein content of the gum (Abdelrahman, 2011). This connection although apparent in the case of ATR gum, is not observed in the case of the other three gums ASG, AMF and ASY, that ASG and AMF (Mohamed Bilal, 2010; Osman, 1993) contain higher protein contents than ASY gum with 1.61% protein content (Hassan, 2000).

3.7. Flow rheological measurements

Fig. 8 shows that all gum emulsions' behavior is nearly Newtonian as the viscosity remained constant at entire shear rate range except for ATR gum emulsion which exhibited shear thinning behavior. ATR gum emulsion showed the highest viscosity followed by AMF, ASG and ASY with the lowest viscosity value among the four gums.

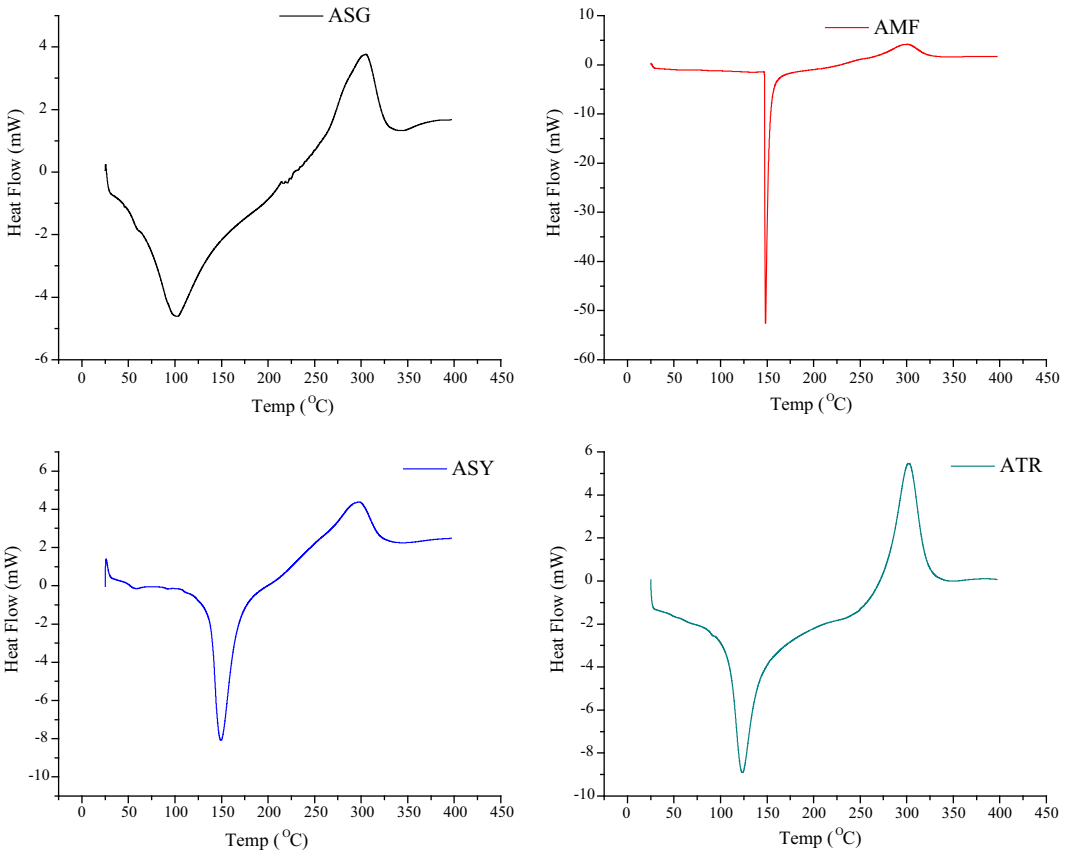

Figure 5 DSC thermogram of ASG, AMF, ASY, and ATR gums.

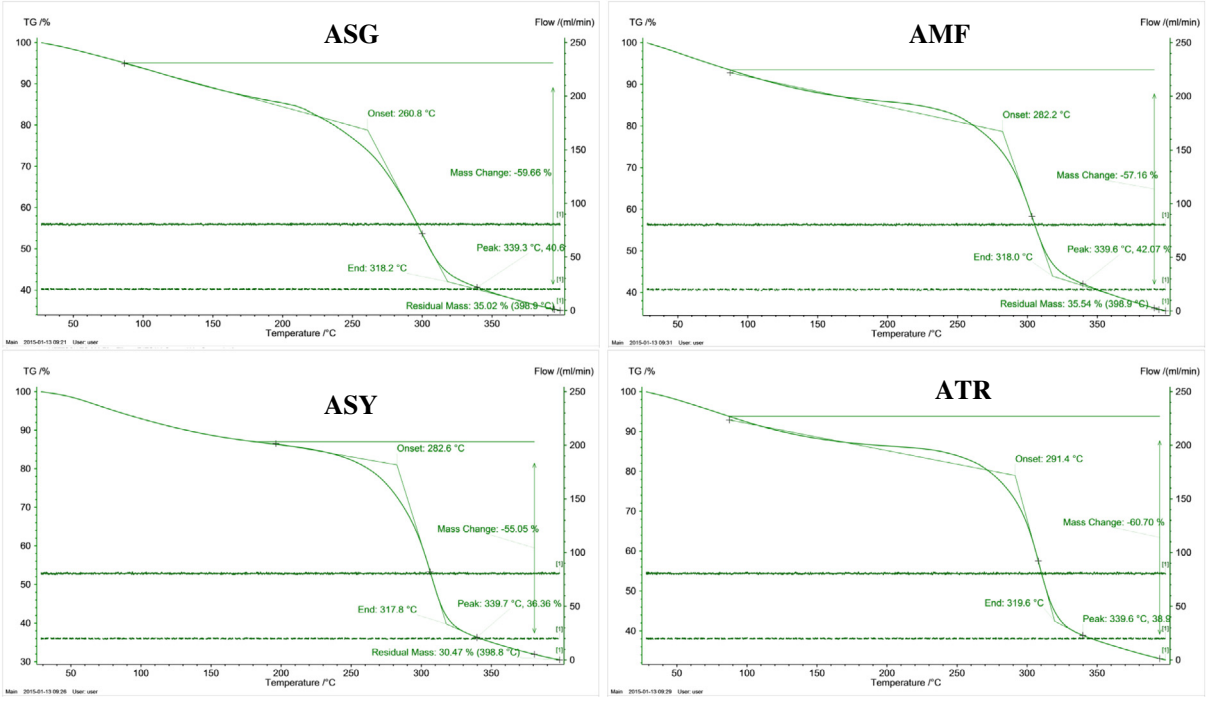

Figure 6 TGA thermogram of ASG, AMF, ASY, and ATR gums.

Table 2 Thermal decomposition data for different samples.

Gum sample	T_0 (°C)[a]	$T_{max.}$ (°C)	% Mass loss
ASG	260.8	339.2	59.66
AMF	282.2	339.6	57.16
ASY	282.6	339.7	55.05
ATR	291.4	339.6	60.70

[a] T_0: onset temperature.

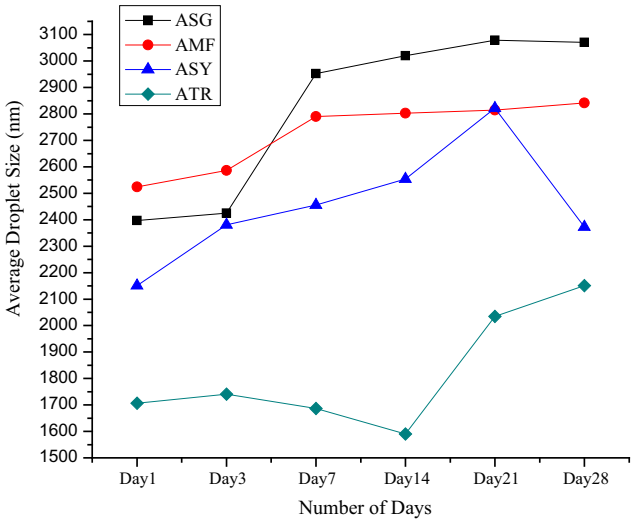

Figure 7 Variation of average droplet size of ASG, AMF, ASY and ATR gum emulsions with time at 20% IPM concentration.

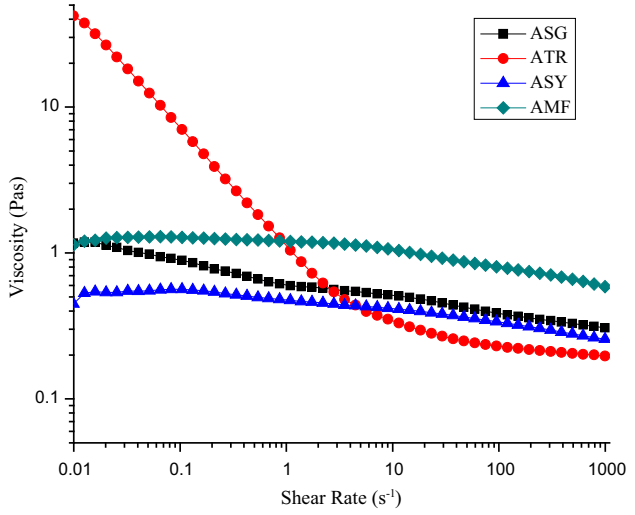

Figure 8 Viscosity profile of 20% IPM *Acacia* gums emulsions at 25 °C.

4. Conclusion

- Although gum samples showed similarities in functional groups, which were clear from the NMR and ATR–FTIR results, the DSC and TGA thermograms were characteristic for each gum and the NMR results reflected different gums structures evident from the characteristic spectrum for each gum.
- Also the gums showed different physicochemical properties reflecting the variations between the *Vulgares* and *Gummiferae* series, and further the variations between the species of the genus *Acacia*.
- The emulsion prepared using ATR gum possessed the smallest oil droplet size and the highest viscosity compared to the emulsions formed with the other gums.

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