Fractionation and Physicochemical Studies of *AcaciaSenegal* Gums

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Abstract: The chemical and physicochemical characteristics of five authenticated A. senegal gum samples from trees of varying age have been investigated. A. senegal was fractionated by a successive non-solvent fractionation technique using ethanol and acetone as a fractionation agent separately. The fractions were found to be different from the whole gum in terms of molecular characteristics. The molecular mass distribution was determined by gel permeation chromatography (GPC-MALLS) with on-line monitoring using light scattering, refractive index and UV absorbance detectors. The result shows that three main component designated arabinogalactane protein (AGP), arabinogalactane (AG) and glycoprotein (GP) known to be present in A. senegal could be present in their fractions. The weight average molecular weight of A. senegal ranged from 0.808 - 1.334 X 10⁶ g/molwith M_w/M_n ranged from (2.89-4.42). The SG4₁₅₋₂₀ sample that from the 10-15 years old tree shows high molecular weight, high viscosity and produce emulsion which were stable under accelerate stress condition. All ethanol fractions and (SG-F1A and SG-F3A) gave the best emulsification performance and stability than the starting material (SG-control) dependent on the protein distribution and its association with the high molecular weight fraction (AGP). All emulsion of ethanol and acetone fractions of A. senegal show low degrees of polydispersity and droplet size compared to the starting material indicating uniformity, clean fractionation and increased adsorption of protein on the oil droplet surface.

Keywords: Acacia senegal, Gum Arabic, Fractionation, Emulsion stability, droplet size, molecular weight

1. Introduction

Gum Arabic is a heterogeneous complex polysaccharide that consists of carbohydrate polymer (composed of galactose (44%), rhamnose (13%), arabinose (27%), glucuronic acid and 4-*O*-methyl glucuronic acid (16%), and small amount of proteinaceous materials (~ 2 %) [1].

The gum from A. senegal has been studied by many workers [2-4] and It has been found to have core β (1 > 3) and (1> 6) linked D-galactopyranose with branches at 0-6 on the (1 > 3)linked residues. The branches consist of L-arabinofuranose, L-arabinopyranose, L-rhamnose and D-glucuronopyranose. It also contains about 2% proteinaceous material which forms an integral part of the structure. The gum consists of a spectrum of molecular species but three distinct components have been recognized following fractionation using hydrophobic affinity chromatography [5].The main component, which corresponds to about 90 % of the total, has a molecular mass and Rh of ~ 2.5×10^5 and ~9 nm respectively and contains very little protein. A second component (~10 % of the total) has a molecular mass and Rh of > 1 x 10^6 and ~23 nm contain ~10 % protein, which comprises mainly of hydroxyproline, proline and serine. It can be degraded by proteolytic enzyme into smaller fragments of molecular mass $\sim 2-3 \times 10^5$ and hence it has been argued that it has a wattle blossom-type structure typical of arabinogalcatan protein complexes, where large carbohydrate blocks (2-3 x 10^5) are attached to a common polypeptide chain [6-8]. Anderson and Stoddart[9] obtained a value of 5 x 10^5 and 8.5 x 10^5 for the number average (Mn) molecular weight using molecular sieve chromatography and for weight average molecular weight (M_w) using light scattering technique for A.senegalgum. Cannoly et al. [10] calculated the molecular mass of the blocks of *A. senegal* gum and found it to be of the order $2x10^5$.Duvallet et al. [11] reported value of $7.2x10^5$ for molecular weight of *A. senegal* gum using low angle laser light scattering technique in IM NaCl at 25.0° C, they also obtained the value of 1.9×10^5 for the number average molecular weight (Mn) using osmometry determination in 0.01M NaCl at 37.0° C. Using GPC-MALLS, Idris et al. [12] obtained values between 2×10^5 and 7.9×10^5 of the weight average molecular weight and values between 1.6×10^5 and 4.5×10^5 of the number average molecular weight and values between 1.6×10^5 and 4.5×10^5 for the weight average molecular weight average molecular weight average molecular weight average Ml to 5.99×10^5 for the weight average molecular weight (Mn) for *A. senegal*gum. Al-Assaf et al. [13] reported a value of 5.99×10^5 for the weight average molecular weight average molecular weight using GPC-MALLS of *A. senegal* and a value of 10.4×10^5 for *A. seyal*.

Polysaccharide hydrocolloids, such as gums, stabilize emulsionsprimarily by increasing the viscosity, slowing the thermodynamically favored breakdown of the emulsion but may also act as emulsifiers, where their emulsion ability is reported as mainly, being due to accompanying, contaminating or intrinsic, protein moieties[14, 15]. In particular, electrostatic interaction between ionic hydrocolloids and proteins may give rise to marked emulsification ability with considerable stability so long as the pH and ionic strength are controlled[16]. Hydrocolloids may interact with other food components such as aiding the emulsification of fats, stabilizing milk protein micelles or affecting the stickiness of gluten. Prakashet al. [5]studied the addition of whey proteins on the functionality of gum Arabic in soft drink emulsions and reported that the addition of small amount whey protein increase in the emulsion activity index. Randall et al. [3] studied the role of the proteinaceous component on the emulsification properties of gum Arabic and reported that only 1-2% of the gum Arabic adsorbed at oil-water interface and that the high molecular mass, protein rich fraction is responsible for the emulsification properties

of gum Arabic as a whole[3]. However the emulsification properties of gums are well reported in literature [17-20].

2. Material and Methods

Acaciasenegal gum samples were collected by one of the authors from Kordofan state, western Sudan. *Acaciasenegal* tree season 2007-2008 of particular ages 5-9, 10-15 and 16-20 years were labeled from $SG1_{5-9} - SG5_{16-20}$ to designate the *A. senegal*gum samples. The subscripted number refers to the ages of the tree.

The physicochemical properties of the gum and fractions such as moisture, ash, specific rotation, intrinsic viscosity, equivalent weight, molecular weight and protein content were determined using standard method[21-23]. Average number molecular weight determination of A. senagalgum samples and their fractions were determined using the GPC system, comprising a high precision HPLC pump (Water, USA), an injector (Rheodyne 7125, Rheodyne, UK), a GPC column (Sepharose 6, Pharmacia, Sweden) attached to a multi-angle laser light scattering system (DAWN DSP, Wyatt Technology USA), UV detector (PyeUnicam, UK), and the software (Astra 4.5 for windows, Wyatt Technology, USA) were arranged. The system is switched on for two hour to equilibrate before carrying out any analysis. Gum samples were accurately weight 0.02g in small vial NaCl to which 5ml of 0.2 M were then added. The vials were stoppered and kept on a roller shaker for two hours. 100microlitre solution was injected into the GPC system via 0.45µm filter (Water Millipore) into the injector fitted with 100-microlitre loop. Elution buffer, 0.2 M NaCl, was passed at flow rate of 0.5 cm³/min at ambient temperature. The gum was fractionated while passing through the Sepharose 6 GPC column and fractions were detected via MALLS, RI and UV detectors responses were collected and analyzed in real time by the computer software [3, 12, 14].

The droplet size of emulsion immediately after preparation (initial) was measured using laser diffraction (Mastersizer 2000, Malven UK). Emulsification effectiveness was evaluated based on the initial particle size of the emulsion. The emulsion stability was evaluated by the change in particle size after acceleration test (3 and 7 days storage at 60 °C) was taken as a parameter to designate the category/ grade of the gum sample.

3. Results and Discussion

3.1. Characterization of *A.senegal*gum and their fractions:

The variation of physicochemical properties of *A. senegal* gum with trees age ranged from 5-20 years old. The highest values of moisture, intrinsic viscosity and acid equivalent weight were that of sample $SG4_{10-15}$ that obtained from 10-15 years old tree. This result coincides with those reported by other Co-workers[25, 26]. Whilst the highest values of specific rotation, number average molecular weight and protein content was obtained by sample $SG1_{5-9}$ that picked from 5-9 years old tree (Table 1).

Sample code	Ash (%)	Moisture (%)	[α] ²⁵ deg	A.E.W	Glucouronic acid	Nitrogen (%)	protein (%)	M _n X 10 ⁵ g/mol	[η] ml/g
SG1 5-9	5.10	11.42	-33°	1135	17.09	0.43	2.86	2.22	19.07
SG25-9	5.23	10.60	-29°	1703	11.39	0.36	2.40	1.63	18.67
SG3 ₁₆₋₂₀	5.07	9.91	-30°	1472	13.18	0.36	2.37	1.96	18.65
SG4 ₁₀₋₁₅	5.06	14.72	-30°	1773	11.17	0.30	1.99	1.16	19.14
SG5 ₁₆₋₂₀	4.00	11.01	-31°	1620	11.96	0.28	1.82	1.02	18.84

 Table 1: Analytical data of the gum exudates from A. senegal samples

The physicochemical properties of ethanol and acetone fractions obtainable by successive solvent fractionation of *A.senegal*gum are given in (Table 2). There is no difference in ash, moisture, optical rotation of all fractions except SG-F2A which show optical rotation (-16°). The protein content of ethanol fraction was much higher than acetone fractions, whilst SG-F2E, SG-F4E and SG-F5E contains protein higher than the starting material. The viscosities of ethanol

fractions are higher than the acetone fractions and less than the viscosity of starting material (SG-control).

The molecular weight was measured for the whole gum as two peaks; the first peak (which corresponds to the AGP) and the second peak (which corresponds to the AG andGP) as identified by the refractive index detector. The summary of the molecular weights parameters of all *A. senegal* samples are given in (Table 3).

Table 2: Analytical	l data of the ethanol	and acetone	fractions of <i>A. senegal</i>	

Fractions	Yield	Ash	Moisture	[α] ²⁵	A. E. W	$M_n X 10^5$	Nitrogen	protein%	Glucouronic	[η] ml/g
Code	%	%	%	deg		g′mol	%		Acid %	
SG-Control		5.41	11.33	-33 °	1711	1.75	0.33	2.21	16.01	19.23
SG -F1E	39.60	5.48	09.75	-25 °	1001	0.041	0.29	1.90	19.37	15.17
SG-F2E	20.50	5.48	11.52	-30 °	521	0.082	0.35	2.28	36.72	14.63
SG-F3E	16.20	5.96	10.73	-23 °	457	0.012	0.34	2.21	41.33	11.98
SG-F4E	04.9	5.63	10.38	-26 °	620	0.041	0.39	2.60	31.29	14.35
SG-F5E	01.20	5.01	12.05	-30 °	218	0.018	0.41	2.73	80.91	14.08
SG -F1A	34.10	5.62	10.80	-28 °	939	0.014	0.32	2.13	20.67	13.76
SG -F2A	19.20	4.55	9.71	-16 °	1708	0.015	0.25	1.67	11.37	13.35

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SG-F3A	08.50	5.83	9.70	-23 °	1502	0.012	0.32	2.11	12.92	11.98
SG-F4A	04.70	6.51	9.07	-27 °	815	0.018	0.29	1.89	23.79	14.39
SG-F5A	01.40	6.13	9.09	-27 °	834	0.032	0.27	1.81	23.26	11.69

Sample code	Mw processed as	%	Rg	M_w/M_n	M _w processed as	%	Rg	M_w/M_n
	one peak	mass	(nm)		two peak	mass	(nm)	
SG1 ₅₋₉	$0.808 \ge 10^{6}$	102	60.1	3.308 ± 0.239	$4.146 \ge 10^6$	12.2	63.2	1.705 ± 0.139
					0.341×10^{6}	87.8	54.2	1.570 ± 0.075
SG2 ₅₋₉	$0.846 \ge 10^{6}$	94.5	54.5	3.604 ± 0.290	9.905 X 10 ⁶	11.0	68.0	1.797 ± 0.109
					$0.335 \ge 10^{6}$	89.0	0.0	1.592 ± 0.113
SG3 ₁₆₋₂₀	$0.966 \mathrm{X} \ 10^{6}$	94.4	65.7	3.730 ± 0.250	3.958×10^6	16.1	75.5	1.522 ± 0.087
					$0.375 \ge 10^{6}$	83.6	37.8	1.705 ± 0.093
SG4 ₁₀₋₁₅	$1.334 \ge 10^{6}$	91.2	55.7	4.920 ± 0.367	6.374 X 10 ⁶	13.8	54.7	1.390 ± 0.040
					0.425 X 10 ⁶	86.2	58.5	1.832 ± 0.144
SG5 ₁₆₋₂₀	0.946 X 10 ⁶	102.9	39.9	2.885 ± 0.092	3.720 X 10 ⁶	15.7	45.3	1.358 ± 0.036
					0.409 X 10 ⁶	84.3	27.7	1.460 ± 0.044

Table 3: Molecular weight parameter of A.senegal samples (GPC-MALLS).

The weight average molecular weights of *A. senegal* samples ranged from 1.335 X 10⁶ to 8.078 X 10⁵ g/molwith the mass recovery 94.5 to 102.6 %. The AGP component was found to be in the range of 11.0 to 15.7 of the total of the gum with the molecular weight range of 3.720×10^5 to 9.905 X 10⁶ g/mol. The result shows that the high molecular weight was found in the sample SG4 ₁₀₋₁₅ that from the 10-15 years old tree. The result agrees with Anderson et al. [28] who noted that the gum from trees between 10 and 15 of age had the highest molecular weight and viscosity; they also noted that the most viscous sample had average nitrogen content, although high nitrogen content did not necessarily imply high viscosity. The molecular weight parameters and

AGP component of ethanol and acetone fractions of *A*. *senegal* are shown in (Table 4). Ethanol fractions of *A*. *senegal* have average molecular weight and AGP component higher than the starting material. The above result indicating that all ethanol fractions have a good emulsifying property and stability compared to the starting material (SG-control) while acetone fraction (SG-F2A, SG-F4A and SG-F5A) have a higher molecular weight and low protein content than the starting material, indicating that these fractions have a poor emulsifying property and stability than the starting material (SG-control).

Table 4: Molecular weight parameter of ethanol and acetone fr	ractions of <i>A.senegal</i> (GPC-MALLS).
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Sample	M _w processed	%	Rg	M _w /M _n	M _w processed as	%	Rg	M _w /M _n
code	as one peak	mass	(nm)		two peak	mass	(nm)	
SG-control	1.135 X 10 ⁶	102.6	32.1	3.697 ± 0.298	4.783 X 10 ⁶	14.1	56.1	1.832 ± 0.071
					$4.264 \ge 10^5$	73.0	24.9	1.626 ±0.211
SG-F1E	$1.708 \ge 10^{6}$	103.6	24.9	3.847±0.690	6.635 X 10 ⁶	20.4	49.8	2.616 ± 0.501
					4.436 X 10 ⁵	79.6	11.9	1.211 ± 0.184
SG-F2E	$1.681 \ge 10^{6}$	116.4	24.4	6.456 ± 0.090	$1.074 \text{ X } 10^{6}$	12.8	49.8	3.821 ± 0.645
					3.527 X 10 ⁵	87.2	17.8	1.535 ± 0.235
SG-F3E	2.209 X 10 ⁶	110.3	27.4	5.133 ± 0.290	$1.086 \ge 10^6$	16.5	50.3	2.821 ± 0.151
					4.948 X 10 ⁵	83.5	20.0	1.352 ± 0.074
SG-F4E	$1.815 \ge 10^{6}$	106.0	23.1	4.227 ± 0.207	8.373 X 10 ⁶	16.7	45.7	2.347 ± 0.117
					4.948 X 10 ⁵	83.4	14.8	1.356 ± 0.046
SG-F5E	1.542 X 10 ⁶	112.3	24.9	4.673 ± 0.213	7.736 X 10 ⁶	15.7	49.0	2.711 ± 0.121
					3.905 X 10 ⁵	84.7	16.9	1.378 ±0.050
SG-F1A	1.216 X 10 ⁶	114.1	60.0	3.409 ± 0.131	5.579 X 10 ⁶	15.3	69.2	1.720 ± 0.062
					4.252 X 10 ⁵	84.7	27.8	1.384 ± 0.044
SG-F2A	$1.262 \ge 10^6$	107.6	83.4	5.507 ± 0.376	11.22 X 10 ⁶	8.4	95.8	3.044 ± 0.242
					3.494 X 10 ⁵	91.7	20.4	1.656 ± 0.055
SG-F3A	$1.432 \ge 10^6$	105.5	85.0	3.984 ± 0.253	8.258 X 10 ⁶	11.6	67.2	1.750 ± 0.066
					4.841 X 10 ⁵	88.4	116.9	1.520 ± 0.152
SG-F4A	1.783 X 10 ⁶	117.0	78.2	4.174 ± 0.200	7.963 X 10 ⁶	17.4	83.7	2.191 ± 0.087
					4.832 X 10 ⁵	82.7	55.4	1.341 ± 0.069
SG-F5A	1.848 X 10 ⁶	108.0	88.7	4.719 ± 0.247	8.725 X 10 ⁶	16.9	95.6	2.716 ± 0.122

The GPC elution profiles of *A. senegal* are shown in (Fig. 1). The RI profile indicates the *A.senegal* samples consisted with two main molecular species. The first one corresponding to the minor peak which appeared at the elution volumes of ~ 7.5 ml represented about 13% of the total mass. The second one corresponding to the major peak which appeared at the elution volume of ~12.5 ml (~ 87 %) of the total mass. The overlay of the elution chromatograms

of *A.senegal* measured by light scattering detector (Fig. 2) illustrate increasing in molecular weight of the sample $SG4_{10-15}$ which obtanied from 10-15 years old tree. The elution profiles obtained by UV(Fig. 3), are very different from the refractive index profile and show three or four molecular mass species for all *A.senegal* samples. Two of the peaks correspond to peak 1 and 2 observed by refractive index and the relative intensity of each is very

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different..However, the elution chromatograms maesured by the refractive index (Fig. 4) is an evident that there is a decrease in the amount of the AG component in the sample $SG4_{10-15}$, which link to give an increase amount of AGF component.

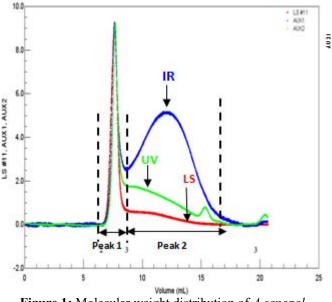


Figure 1: Molecular weight distribution of *A.senegal* SG4₁₀₋₁₅

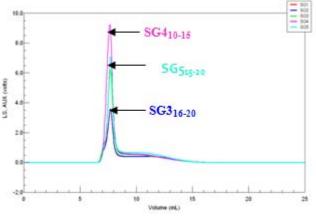


Figure 2: GPC elution profiles of *A.senegal* using light scattering.

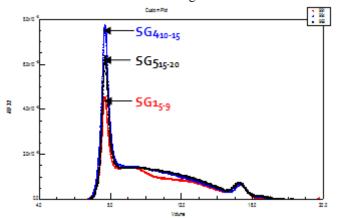


Figure 3: The elution profiles monitored by UV at 214 nm for the *A.senegal* samples.

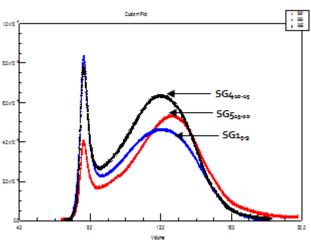


Figure 4: The elution profiles monitored by RI at 214 nm for the *A. senegal* samples.

3.2. Emulsification properties and stability of *A.senegal* and their fractions.

Emulsification effectiveness was evaluated based on the initial particle size of the emulsions. The emulsions were then subjected to an acceleration testing (stored for 3 days and 7 days at 60 °C). Particle size diameter of emulsion after the acceleration test was measured using a particle size distribution analyzer. Emulsification stability was evaluated by the change in particle size of emulsion after acceleration test (stored for 7 days at 60 °C) was taken as a parameter to designate the category of the gum sample. Therefore, the gum samples which showed a change of 0.1 μ m or less were classified as category 1 status (good emulsifier). A change >0.1–1.0 μ m were classified as category 2 status. The less stable emulsions which showed a change >1.0 μ m were allocated category 3 status (poor emulsifier).

The result shows that the less change in particle size diameter was found in $SG4_{10-15}$ sample that picked from 10-15 years old tree. For this reason they can be allocated a grade 1. On the other hand the volume median diameter of $SG1_{5-9}$ and $SG2_{5-9}$ sample from 5-9 years old tree increased to 200% and 160% over the initial volume respectively. Therefore the $SG1_{5-9}$ and $SG2_{5-9}$ did not show good stability and gave a high change in particle size diameter (0.58 and 0.29µm), for this reason allocated a grade 2 status (less stable emulsifier)(Table 5 and Fig. 5).

The volume median diameter of $SG3_{16-20}$ and $SG5_{16-20}$ sample from 16-20 years old tree increased to 122% and 108% over the initial volume respectively. Also the result indicates that the change in particle size diameter of $SG3_{16-20}$ and $SG5_{16-20}$ was 0.098 and 0.045µm respectively. For this reason they can be allocated a grade 1 status, but of less stability than $SG4_{10-15}$ sample. The less change in particle size diameters were found in the sample $SG4_{10-15}$ which obtained from 10-15 years old tree, indicating the increase adsorption of the protein on the oil droplet surface and maximum the protein/surface contact and hence reduce the droplet size. This might be promoted by a high viscosity of the continous phase, giving enough time for protein to be adsorbed and thus stabilizes the oil droplet surface from coalescence [27].

All ethanol fractions have a less change in particle size than 0.1 μ m, for this reason they can be allocated grade 1 status compared to grade 2 status for the starting material (SG-control) (Table 5) and (Fig. 6), these results are largely explained by the association of protein with high molecular fraction AGP as shown in (Fig. 7).

Table 5: Particle size diameter of A.senegal emulsions using	5
a laser diffraction particle size analyzer.	

	a fuser affinaction particle size analyzer.									
Samples	Grade	Initial VMD	ATST VMD	ESI (µm)						
code		(µm)	(µm)							
SG-control	2	0.598	0.704	0.196						
SG1 5-9	3	0.452	1.128	0.676						
SG25-9	2	0.475	0.784	0.289						
SG3 ₁₆₋₂₀	1	0.437	0.535	0.098						
SG4 ₁₀₋₁₅	1	0.503	0.526	0.023						
SG5 ₁₆₋₂₀	1	0.551	0.596	0.045						
SG-F1E	1	0.575	0.639	0.064						
SG-F2E	1	0.522	0.572	0.050						
SG-F3E	1	0.844	0.890	0.046						
SG-F4E	1	0.680	0.75	0.070						
SG-F5E	1	0.545	0.642	0.098						
SG-F1A	1	0.696	0.718	0.022						
SG-F2A	2	0.974	1.336	0.362						
SG-F3A	1	0.767	0.817	0.050						
SG-F4A	2	0.755	0.863	0.108						
SG-F5A	2	0.700	0.841	0.141						

*ATST means Accelerated temperature stress test for 7 days at 60 °C. *ESI means Emulsion Stability Index.

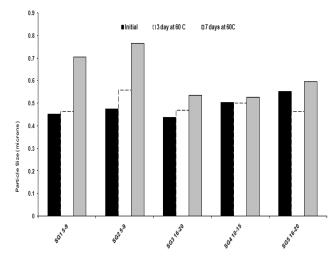
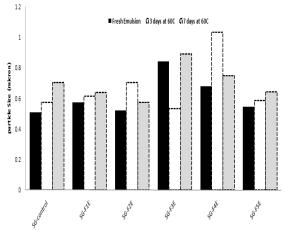
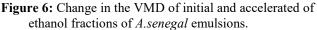


Figure 5: VMD of initial and accelerated of *A.senegal* emulsions.

Acetone fraction (SG-F1A and SG-F3A) gave the best performance and stability and was significantly better than the starting material (SG-control) (Fig. 8). Fraction (SG-F1A and SG-F3A) showed the high UV response compared to the starting material (Fig. 9) indicating the protein is associated with high molecular weight fraction (AGP). The results showed that it's possible to produce good and stable emulsifier fraction from less emulsifier when the protein distribution is changed and shift to the high molecular fraction (AGP).





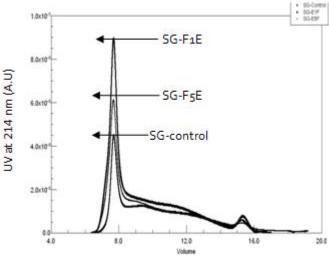


Figure 7: UV profile at 214 nm of *A. senegal* control and fractions obtained in ethanol.

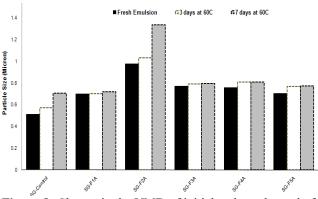


Figure 8: Change in the VMD of initial and accelerated of ethanol fractions of *A. senegal* emulsions.

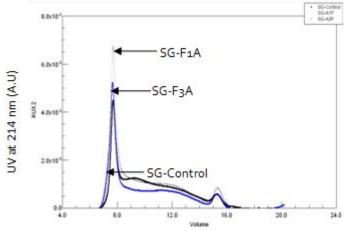


Figure 9: UV profile at 214 nm of *A. senegal* control and fractions obtained in Acetone.

4. Conclusion

Analytical parameters gave different data for the various *A.senegal* and fractionated samples and these include yield, moisture, ash, pH, specific rotation, intrinsic viscosity, nitrogen and protein contents. The results in this study showthe differences between *A.senegal* and their fractions and confirm the role of high molecular weight fraction (AGP) as the fraction responsible for the emulsification performance and stability.

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