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RESEARCH ARTICLE

PHYSICOCHEMICAL CHARACTERIZATIONS OF ACACIA SIEBERANA VAR. SIEBERANA OF SUDANESE ORIGIN.

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Abstract

Twenty four authentic samples of Acacia sieberana var. sieberana were collected from South Kordofan (SK) and White Nile (WN) states. The samples were analyzed to determine average values, of moisture, ash, pH, specific rotation, refractive index, number average molecular weight, intrinsic viscosity, nitrogen content, protein content, total uronic acid and acid equivalent weight of the gum. For (SK)samples average values, for the above parameters, were found to be: 8.56 %, 1.61%, 4.34, +104°, 1.337, 2.13×10^6 Da, 8.56 ml /g, 0.37%, 2.45%, 9.2% and 1933 respectively, while those of WN samples were found to be: 9.12 %, 1.65%, 4.38, 103, 1.337, 2.29×10^6 Da, 9.39 ml /g, 0.37 %, 2.43%, 9.4 % and 1884 respectively. The cationic composition showed the following sequence: calcium > potassium> magnesium > sodium. Traces of: Fe, Ni, Co, Mn, Cu and Pb were detected. Percent average values of sugar composition show that galactose, arabinose, rhamanoe, glucuronic acid and 4-O-methylglcuronic acid for the gum samples from both locations are similar. For SK samples the values were found to be: 9.8, 57, 4.0, 2.7 and 6.5 respectively, while those of WN samples were found to be: 9.9, 56, 3.1, 2.4 and 6.6 respectively. The molecular weight distributions, of two composite samples, of the gum from the two locations indicate the presence of three main fractions. The weight average molecular weight of SK and WN, composite samples, is 1.8×10^6 Da and 1.6×10^6 Da respectively. The emulsifying capacity study of the gum shows that the gum is a grade one emulsifying agent.

Introduction:-

Polysaccharide gums derived from varieties of Acacia species are used extensively in food and pharmaceutical industries (Millard and Balmert 1961; Tame-Said 1997). Although many Acacia species produce gums of potential quality, the varieties of marketable gums are limited. Considering the scarcity of gums during the last few years, it became important to explore new sources of gums and evaluate their potential application qualities. However, Sudan provides an ideal location for such exploratory studies, as it possesses a diversity of natural forests including species from non acacia resources (Sahni, 1968).

Acacia sieberana has been classified, taxonomically, as Gummiferae, Bentham's Series 4 (Bentham, 1875). Limited studies on the properties of the gum were reviewed by Adriaens, 1939 and Anderson et al., 1973. Karamalla, 1999 reported some analytical data for three samples of sieberana species, namely A. sieberana var. sieberana, A. sieberana var. vermesenii and A. sieberana var. Villosa. The polysaccharide from Acacia sieberana var. sieberana gum is one of the available gum resources which are not exploited commercially.

The present work is an attempt to extend the scope of previous studies and to establish a frame of specifications for the gum from this species taking in consideration international guidelines for the specification for marketable gums from Acacia. It was also intended to investigate the effect of environmental variations on the gum quality. The work also was aimed to investigate some functional properties of the gum from this species.

Materials and methods:-

Materials:-

Gum samples were collected from (SK and WN) states in seasons 2006-2008 and authenticated by botanist of Ministry of Forestry-Sudan. The samples were cleaned from sand and bark impurities. Representative samples were powdered to a white-pale yellow powder using a pestle and mortar.

Methods:-

Standard methods of analysis to determine physicochemical properties were used (Chickamai et al., 1996). The gel permeation chromatography (GPC) method coupled to a multi-angle laser light scattering detector, a refractive index detector and a UV detector operated at 214 nm used in this study has been previously described (; Al-Assaf, et al., 2005).

The emulsification function is investigated by measuring the droplet size distribution of the emulsion at three different temperatures: as fresh sample and after storage for 3 and 7 days once at ambient temperature and once at 60 ^{o}C (using accelerated stability test). The samples were subjected to Mastersizer 2000, a laser diffraction particle size analyzer (Malvern Instruments). Distilled water was used as dispersant and a value of 1.450 was used for the refractive index for oil phase Octanoic/Decanoic acid triglyceride oil (ODO) (Katayama, et al, 2006).

Emulsion preparation:-

Distilled water was added to about 12 g of the gum sample (based on dry weight) in glass bottle to become about 40 g in total with a concentration of 30 % (w/w) gum solution. The sample was agitated on a tube roller mixer overnight until the sample completely dissolved and hydrated. Exact calculated grams for each samples (~27 - 28 g) of the prepared gum solution was filtered using 100 µm mesh then mixed with 0.52 ml of 10 % (W/V) sodium benzoate solution as a preservative, and 0.48 ml of 10 % (W/V) citric acid solution to adjust the pH to 4, distilled water was added until the total weight become 32.0 g. then, 8.0 g ODO oil was added to the gum solution to give a total of 40 g and final concentration of 20%. The mixed solution was homogenized for 3 minutes using a polytron (PT-2100) homogenizer at 26000 rpm. Impeller (PTDA21 9 mm tip diameter) was used as dispersing tool. To achieve small particle size < 1 micron, the pre-emulsified mixture was homogenized using a high-pressure Nanomizer (NM2-L100, Yoshida a kikai Co. Ltd.). In order to achieve effective disaggregation of the gum it was passed twice at 50 M Pa. The final emulsion was divided into two aliquots and kept in closed glass universals. One of the aliquots was kept at 60 oC in the Vacuum Oven (Gallenkamp. OVA031.XX1.5), and the other was kept at ambient temperature (Sakata et al, 2006).

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Results and discussion:-

Tables (1 and 2) show the results of the physicochemical characterization of the samples from SK and WN states. Average values for % moisture, % ash, %nitrogen, % protein, % total uronic acid pH, specific rotation, number average molecular weight, intrinsic viscosity, and equivalent weight are almost identical for all samples, however very slight variations are observed.

Low intrinsic viscosity indicates a highly branched globular structure (Barrow, 1979). Percentage of nitrogen and protein for SK and WN samples are three times that of Acacia seyal (Hassan et al., 2005) and are in the same order for those of Acacia senegal (Osman et al., 1995). Eequivalent weights for SK and WN samples are slightly different and are higher than the values reported for Acacia seyal (Hassan et al., 2005). The high equivalent weight for Acacia sieberana var. sieberana may be attributed to its high molecular weight and relatively low uronic acid content.

Tables (3 and 4) show the major cationic compositions of Acacia sieberana var. sieberana gum samples from SK and WN respectively. The results indicate that the major elements are in the order Ca > K > Mg > Na for SK samples and in the order Ca > K > Na > Mg for WN samples. These differences may be attributed to differences in soil element composition.

Tables (5 and 6) show the sugar compositions of Acacia sieberana var. sieberana gum for SK and WN samples respectively. The results indicate two facts: arabinose: galactose ratio is >1 and rahmnose Content is low. These two facts are typical features of gums from the Gummeferae (Anderson and McDougall, 1987).

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Table 1:- Physicochemical data of A. sieberana var. sieberana collected from South Kordfan state.

Sample code	**SK_1**	**SK_2**	**SK_3**	**SK_4**	**SK_5**	**SK_6**	**SK_7**	**SK_8**	**SK_9**	**SK_{10}**	**SK_{11}**	**SK_{12}**	**Mean**	**SD**	**CV %**
Moisture %	8.19	8.99	8.92	7.31	9.02	8.54	9.02	7.65	9.01	9.18	7.86	9.06	8.56	0.65	7.55
Ash %	1.35	1.33	1.42	1.41	1.50	1.72	1.95	1.86	1.84	1.92	1.57	1.48	1.61	0.232	14.4
pH	4.25	4.48	4.23	4.25	4.50	4.30	4.50	4.45	4.52	4.37	4.26	4.38	4.37	0.11	2.58
Specific rotation	101	102	102	104	108	106	104	104	106	102	101	103	104	2.2	2.12
Refractive index	1.338	1.337	1.338	1.338	1.337	1.336	1.337	1.337	1.337	1.337	1.337	1.337	1.337	0.0006	0.05
M_n ($\times 10^6$ Da)	2.52	2.10	1.78	1.97	1.85	2.68	2.05	1.95	2.05	2.10	2.34	2.12	2.13	0.26	12.5
Intrinsic viscosity	9.6	9.6	11.7	5.3	7.6	8.3	7.8	9.2	8.6	9.1	7.8	8.1	8.56	1.53	17.9
Nitrogen %	0.37	0.39	0.32	0.38	0.37	0.36	0.38	0.35	0.37	0.39	0.42	0.39	0.37	0.02	6.6
Protein %	2.44	2.57	2.11	2.51	2.44	2.38	2.51	2.31	2.44	2.57	2.77	2.37	2.45	0.16	6.6
Uronicacid anhydride %	9.2	9.4	7.8	8.6	10.6	9.7	9.7	10.5	9.3	7.3	9.2	9.2	9.2	1.0	10.4
Equivalent weight	1914	1873	2257	2048	1656	1816	1816	1677	1894	2413	1914	1914	1933	218	11.3

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Table 2:- Physicochemical data of A. sieberana var. sieberana collected from White Nile state

Sample code	**WN_1**	**WN_2**	**WN_3**	**WN_4**	**WN_5**	**WN_6**	**WN_7**	**WN_8**	**WN_9**	**WN_{10}**	**WN_{11}**	**WN_{12}**	**Mean**	**SD**	**CV %**
Moisture %	8.19	8.99	8.92	9.11	9.02	9.45	9.20	9.15	9.52	9.48	8.95	9.45	9.12	0.37	4.016
Ash %	1.45	1.38	1.42	1.41	1.48	1.65	1.78	1.64	1.92	1.72	1.91	1.98	1.65	0.22	13.26
pH	4.35	4.48	4.23	4.25	4.50	4.25	4.52	4.44	4.60	4.24	4.38	4.28	4.38	0.13	2.94
Specific rotation	101	104	102	101	99	104	106	100	108	104	105	102	103	2.63	2.55
Refractive index	1.338	1.337	1.338	1.338	1.337	1.337	1.338	1.338	1.337	1.336	1.337	1.337	1.337	0.001	0.05
M_n ($\times 10^6$ Da)	2.52	2.10	2.68	2.38	2.05	2.45	2.16	2.60	2.02	1.95	2.40	2.24	2.30	0.24	10.6
Intrinsic viscosity	9.1	10.2	11.7	8.6	7.4	8.3	7.6	6.8	9.4	11.2	10.6	11.8	9.39	1.71	18.2
Nitrogen %	0.35	0.37	0.39	0.38	0.39	0.34	0.38	0.36	0.39	0.38	0.35	0.34	0.37	0.02	5.3
Protein %	2.31	2.44	2.57	2.51	2.57	2.24	2.51	2.38	2.57	2.51	2.31	2.24	2.43	0.13	5.3
Uronic acid anhydride %	9.8	9.8	7.2	9.6	10.2	8.8	9.2	9.6	10.4	9.6	9.5	9.4	9.4	0.8	8.7
Equivalent weight	1797	1797	2446	1834	1727	2001	1914	1834	1693	1834	1853	1874	1884	194	10.3

Table 3:- The major cationic content in ppm, of Acacia sieberana var. sieberana gum (SK).

Le pmaS code	aN	K	aC	gM	eF	iN	oC	nM	Cu	bP
SK_1	624	1600	13663	1176	28	100	0	7	1	3
SK_2	580	1250	12601	375	10	106	4	6	2	4
SK_3	341	1430	17301	434	17	89	0	9	0	2
SK_4	342	2080	14842	773	18	78	6	4	2	0
SK_5	423	1800	12997	821	15	76	1	5	3	4
SK_6	284	2443	22082	780	21	86	0	2	2	3
SK_7	521	852	16430	648	19	85	0	3	3	0
SK_8	262	1210	20150	268	23	94	4	9	0	2
SK_9	282	1480	15410	440	19	68	1	5	2	0
SK_{10}	425	945	12105	283	14	64	3	4	1	4
SK_{11}	228	1355	10285	329	17	73	0	7	3	5
SK_{12}	342	1284	15200	420	16	82	5	5	0	4
Mean	388	1477	15256	562	18.1	83.4	2	5.5	1.6	2.6
SD	129	454	3380	278	4.6	12.6	2.3	2.2	1.2	1.8
CV%	33.2	30.7	22.2	49.4	25.3	15.1	112.8	39.9	73.5	69.0

SD =standard deviation; CV = coefficient of variation

Table 4:- The major cationic content in ppm, of Acacia sieberana var. sieberana gum (WN).

Sample code	aN	K	aC	gM	eF	iN	oC	nM	uC	bP
WN_1	1500	5012	15712	420	28	190	5	16	7	3
WN_2	850	4109	16840	280	27	210	3	12	2	9
WN_3	1025	3025	13500	580	8	200	3	10	6	6
WN_4	1400	2758	12280	846	45	225	2	12	4	4
WN_5	425	4400	15000	850	38	275	5	8	5	0
WN_6	1075	2860	13100	825	15	290	3	6	5	7
WN_7	736	2005	10284	480	9	80	2	7	2	6
WN_8	628	2443	8484	158	11	82	1	6	7	4
WN_9	961	1214	17411	176	12	79	1	9	2	0
WN_{10}	1198	1042	20152	325	18	96	0	4	6	3
WN_{11}	1661	8513	16433	344	15	109	1	3	5	4
WN_{12}	1676	2722	20084	326	16	115	2	2	4	3
Mean	1095	3342	14940	467.5	20	163	2.3	7.9	4.6	4.1
SD	405	2016	3594	253	12	78	1.6	4.1	1.8	2.6
CV%	37.0	60.3	24.1	54.1	58.7	48.0	66.7	52.1	40.0	64.8

SD =standard deviation; CV = coefficient of variation

Table 5:- The sugar composition of Acacia sieberana var. sieberana gum (SK).

Sample code	Galactose%	Arabinose%	Rhamanose%	Glucuronic acid %	4-O-methylglcuronic acid %
SK_1	10	58	4.6	2.6	6.6
SK_2	11	56	5.3	0.0	9.4
SK_3	9	60	3.8	2.8	5.0
SK_4	9	59	4.1	3.0	5.6
SK_5	12	52	2.8	4.6	6.0
SK_6	10	54	3.9	5.2	4.5
SK_7	8	58	4.6	0.0	9.7
SK_8	9	57	0.0	3.4	7.1
SK_9	8	59	2.5	0.0	9.3
SK_{10}	11	62	6.0	1.9	5.4
SK_{11}	8	57	5.2	3.6	5.6
SK_{12}	12	54	4.8	5.6	3.6
Mean	9.8	57	4.0	2.7	6.5
SD	1.5	2.8	1.6	1.96	2.0
CV%	15.2	4.9	40.4	71.9	31.1

SD =standard deviation; CV = coefficient of variation

Table 6:- The sugar composition of Acacia sieberana var. sieberana gum (WN).

Sample code	Galactose%	Arabinose%	Rhamanose%	Glucuronic acid %	4-O-methylglcuronic acid %
WN_1	12	57	5.1	4.5	5.3
WN_2	9	63	3.4	1.8	8.0
WN_3	10	61	6.0	2.3	4.9
WN_4	11	58	4.2	4.4	5.2
WN_5	9	60	0.0	0.0	5.6
WN_6	11	43	2.7	0.0	8.8
WN_7	9	57	0.0	2.5	6.7
WN_8	10	48	4.5	3.0	6.6
WN_9	8	56	3.4	3.2	7.2
WN_{10}	9	57	0.0	2.8	6.8
WN_{11}	10	54	3.8	1.6	7.9
WN_{12}	11	56	4.2	3.1	6.7
Mean	9.9	56	3.1	2.4	6.6
SD	1.2	5.5	2.1	1.4	1.2
CV%	11.7	9.9	66.1	58.9	18.4

SD =standard deviation; CV = coefficient of variation

Figures: 1 and 2 show the GPC elution profiles for SK and WN respectively. The profiles reveal the presence of three main fractions differing very much in their molecular weight, emphasizing the polydispersity of the gum. These fractions resemble the arabinogalactan-proteins (AGP), arabinogalactan (AG) and glycoprotein (GP) described for Acacia senegal (Randall et al, 1988). However, the distribution patterns of these fractions are slightly different indicating different detailed structure of the three components.

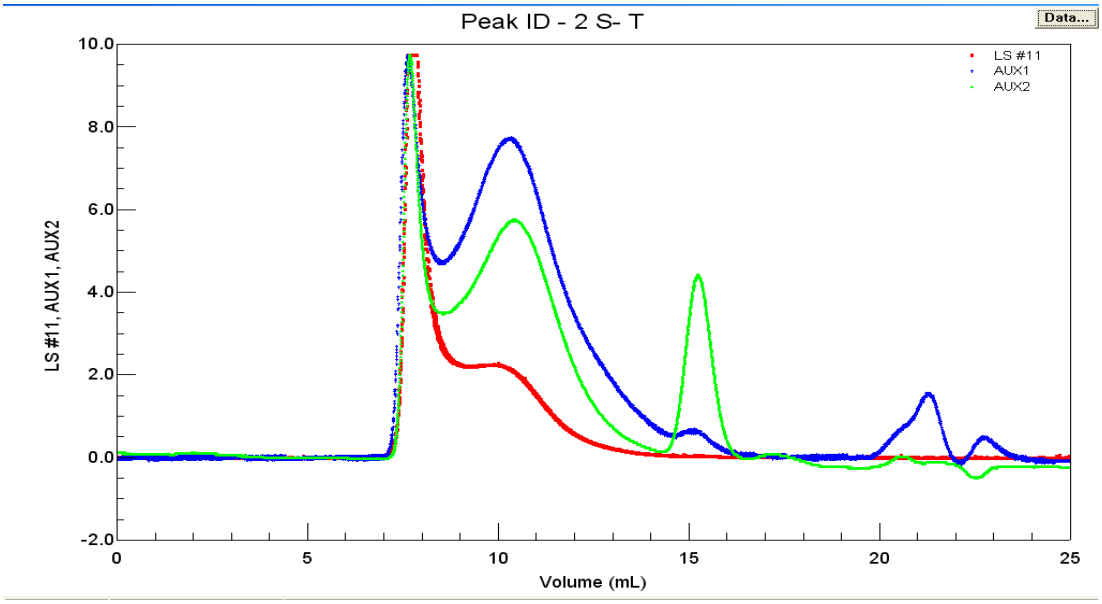

Fig. 1:- GPC elution profiles of A. sieberana var. sieberana gum (SK).

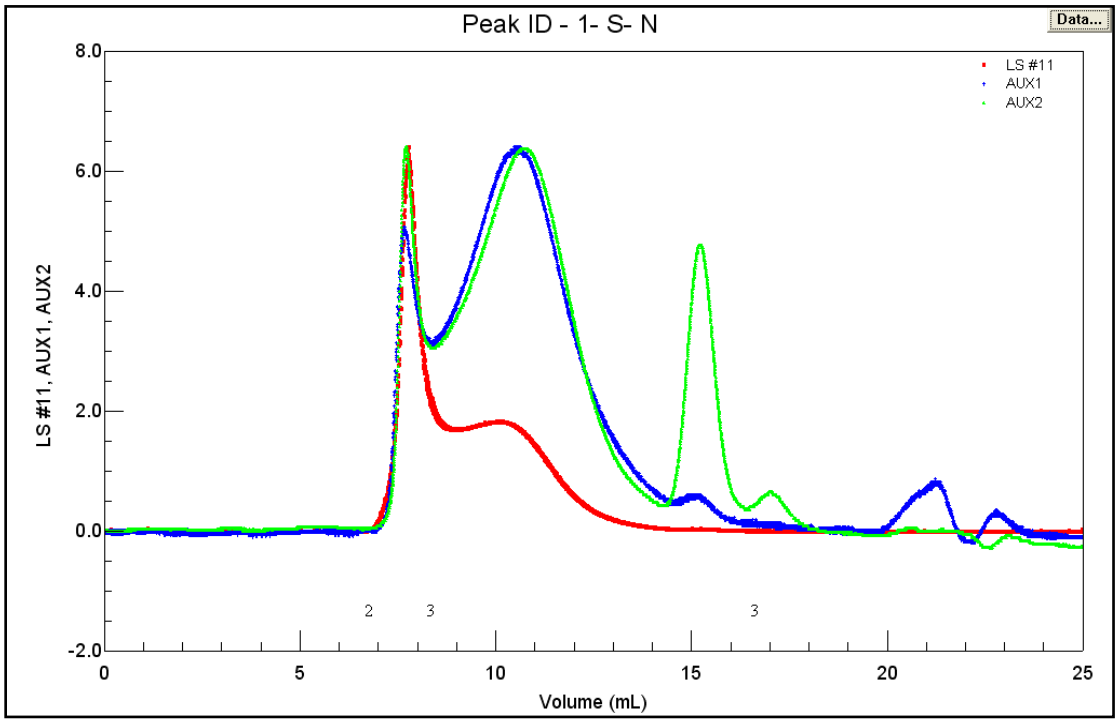

Fig. 2:- GPC chromatogram showing the elution profiles monitored by light scattering (red), refractive index (blue) and UV (214 nm, green) detectors for A. sieberana var. sieberana gum (WN).

Table 7 also shows the weight average molecular weight for the gums from A.sengal, A. seyal and A. sieberana var. siebarana. The values for the first fraction for all gums are slightly different (eluted at almost the same volume). For the second fractions, for all gums the values are quite different indicate different retention time and hence different molecular weights. A. sieberana var. siebarana, from both locations, has the highest value of molecular weight followed by A.seyal and A. Senegal (Fig .3).

Table 7:- Molecular weight parameters and root mean square radius of gyration (R_g) of A. sengal, A.seyal and A.siebarana var. sieberana (two locations) determined by GPC-MALLS.

Sample code	M_w processed as one peak	R_g (nm)	M_w/M_n	M_w processed as two peaks	R_g (nm)	M_w/M_n
A. sengal Control	1.1×10^6	32.1	3.7 ± 0.3	4.5×10^6	56.1	1.8 ± 0.0
				4.2×10^5	24.9	1.6 ±0.2
A .seyal Control	1.7×10^6	26.6	2.4 ± 0.2	4.7×10^6	37.6	1.4 ± 0.1
				8.9×10^5	22.3	1.5± 0.2
A. sieberana (SK)	1.8×10^6	22.3	1.2 ± 0.1	5.1×10^6	41.1	1.7±0.1
				1.1×10^6	10.9	1.3±0.1
A. sieberana (WN)	1.6×10^6	19.1	1.7±0.1	4.8×10^6	38.3	1.1±0.0
				1.0×10^6	13.2	1.3±0.1

Note: The high molecular weight peak was processed separately and the remainder of the gum processed as the second peak; M_w= weight average molecular weight; M_n= number average molecular weight.

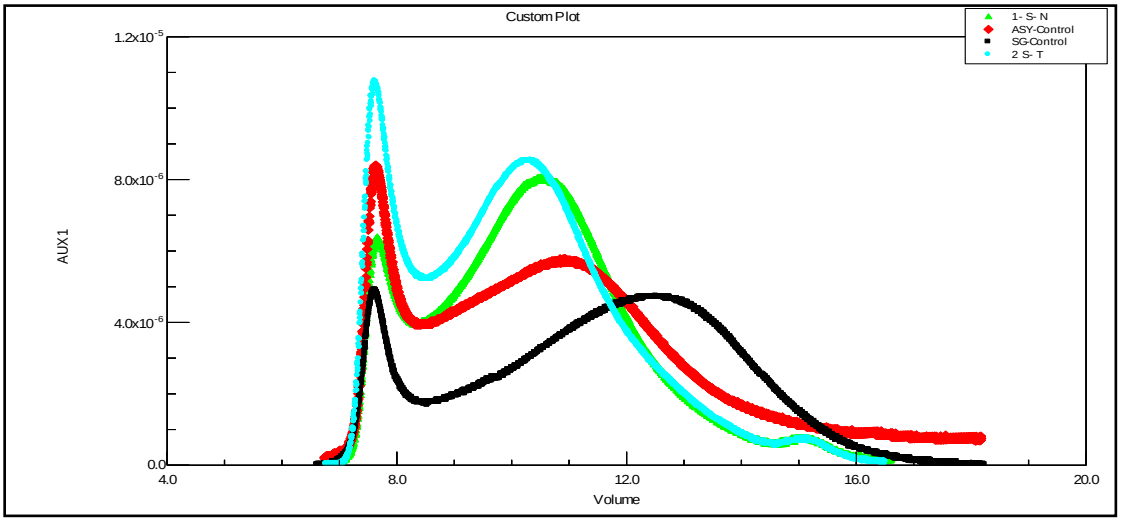

Fig. 3:- Comparison of the elution profiles monitored by refractive index detector for the gums: A. senegal (black), A. seyal (red), A. sieberana var. sieberana (SK) (violet) and A. sieberana var. sieberana (WN) (green).

Figures 4 and 5 show Particle size distributions of A. sieberana var. sieberana (SK) and (WN) emulsions, all particle sizes are almost less than (~1 micron) and the changes in the distributions with time and temperature for the gum from both locations are appreciably small, indicating good emulsification properties.

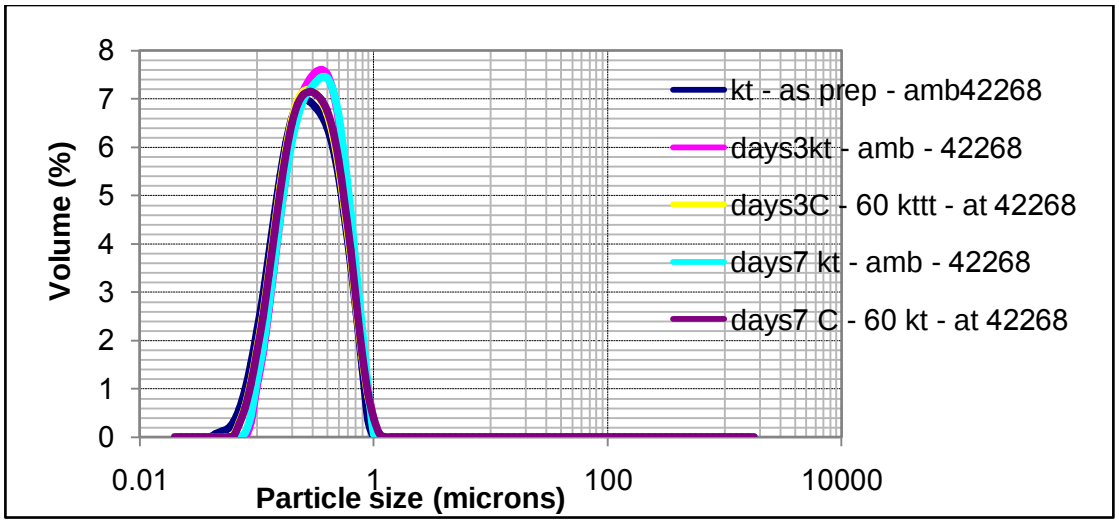

Fig. 4:- Particle size distributions of A. sieberana var. sieberana (SK) emulsion different conditions.

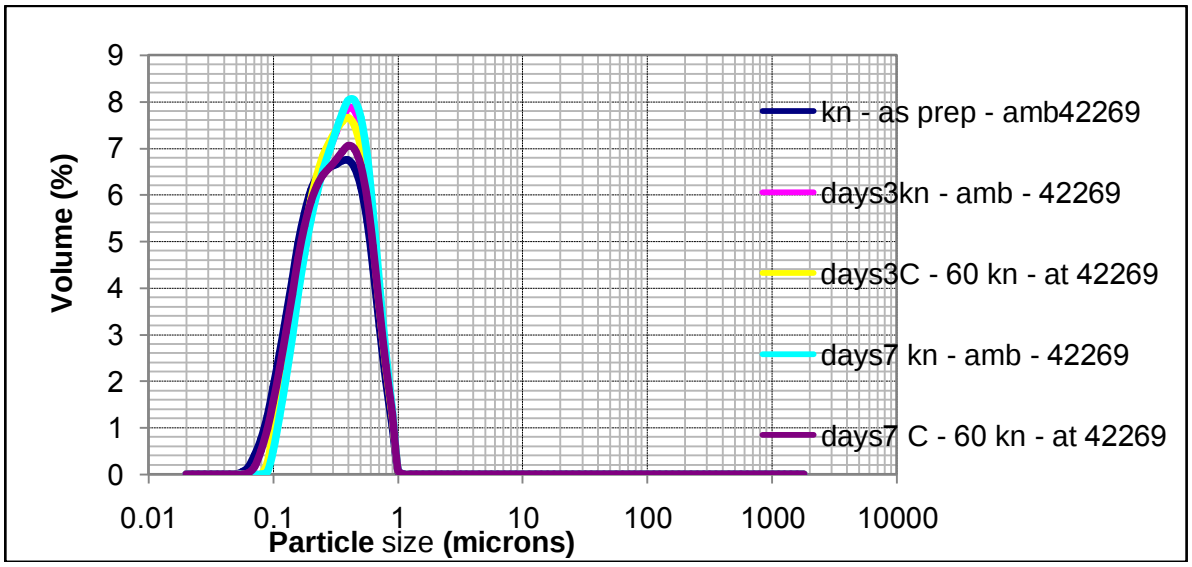

Fig. 5:- Particle size distributions of A. sieberana var. sieberana (WN) emulsion different conditions.

Summary and conclusions:-

The physicochemical study of Acacia sieberana var. sieberana gum shows the followings characterizations: positive optical rotation, low viscosity and low rhaminose content. The GPC fractionation of this polysaccharide gum shows the presence of three fractions and the protein is distributed among all these fractions with considerable ratios. From a functional application view, the gum is considered a grade one emulsifier.

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References:-

1. Al-Assaf, S., Phillips, G.O., Peter A. William, P.A. (2005): Studies on acacia exudate gums. Part I: The molecular weight of Acacia senegal gum exudates, Food Hydrocolloids, **19**, 647–660.
2. Anderson, D. M. W., BELL, P. C., Conant, G. H. and McNab, C. G. A.: The gum exudates from Acacia dealbata and Acacia sieberana (Correction to previous analytical data), (1973) Carbohydrate Res., **26**, 99-104.
3. Anderson, D.M.W. (1986): Food Addit. Contam., **3**, 225.
4. Anderson, D. M. W., and McDougall, F. J. (1987): The composition of the proteinaceous gum exuded by acacia gerrardii and acacia goetzii subsp Goetzii. Food Hydrocolloids, **1**, 327–331.
5. Barrow, G. M. (1979): In Textbook of Physical Chemistry, Mc GRAW-HILL book company, New York, pp. 762-764.
6. Bentham, G. (1875): Trans. Linn. Soc, (London), **30**, 444.
7. Chikmeai, B. N., Phillips, G. O., and Casedai, E. (1996): The Characterization and Specification of Gum Arabic, FAO, Rome, Technical Cooperation Programme, Project No: TCP/RAF/4557.
8. FAO, Rome (1990): Food and Nutrition paper No. **49**, 23.
9. Fincher, G. B., Stone, B. A., Clarke, A. E. (1983): Arabinogalactan proteins: structure, biosynthesis and function, Annu Rev Plant Physiol, **34**, 47–70.
10. Hassan, E.A, Al-Assaf, S., Phillips, G.O., and Williams P.A. (2005): Studies on Acacia gums: Part III molecular weight characteristics of Acacia seyal var. seyal and Acacia seyal var. fistula, Food Hydrocolloids **19**, 669–677.
11. Idris, O. H. M., Williams, P. A., & Phillips, G. O. (1998): Characterization of gum from Acacia senegal trees of different age and location using multi detection gel permeation Chromatography. Food Hydrocolloids, 12, 379–388.
12. Karamalla, K.A., (1999): Analytical data of the gum exudates from different Acacia species of the Sudan, Gums stabilizers for food industry, **10**, 40.
13. Katayama, T., Sasaki, Y., Ogasawara, T., Nakamura, M., Sakata, M. Al-Assaf, S. and Phillips, G. O.,(2006): Estimation of Concentration and Performance of AGPs in Emulsion Systems Using Gum Arabic,Foods food ingredients J, **211**, No. 3, 222-227.
14. Millard R, Balmert CA (1961): Effervescent compositions, US patent application 2,985,562.
15. Osman, M. E., Menzies, A. R., Williams, P. A, Phillips, G.O., Baldwin, T.C. (1993a): The molecular characterisation of the polysaccharide gum from Acacia Senegal, Carbohydr Res **246**,303–318.
16. Osman, M.E., Williams, P.A., Menzies, A.R., Phillips, G.O. (1993b): Characterization of commercial samples of gum arabic, J Agric Food Chem. **41**, 71–77.
17. Osman, M. E., Menzies, A. R., Martin, B. A., Williams, P. A, and Baldwin, T.C. (1995): Characterization of Gum arabic Fractions obtained by Anionic Chromatography, Phytochemistry **38** No.2 407-417
18. Randall, R. C., Phillips, G. O., and Williams, P. A. (1988): The role of the proteinaceous component on the emulsifying properties of gum arabic, Food Hydrocolloids, **2**(2), 131–140.
19. Sahni, K. C. (1968): In Important Trees of the Northern Sudan, Khartoum University Press, Khartoum, Sudan, pp. 58-59.
20. Sakata ,M., Katayama, T., Hirose, Y., Ogasawara, T., Saski,Y., Nakamura,M., Al-Assaf, S. and Phillips, G. O.,(2006): Effect of Pressure Homogenization in Emulsification with AGP, Foods food ingredients J, **211**, No. 3, 230-237.
21. Tame-Said, J. I. (1997): Toothpaste and mouthwash in tablets, Patent application WO 9,719,668.