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Acacia Seyal Characterization and fractionation

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ABSTRACT

Ten Acacia seyal var. seyal samples, of Sudan origin, were characterized by a number of physicochemical methods and fractionated . Physical and chemical properties such as moisture percent, ash percent, intrinsic viscosity, specific rotation, nitrogen and protein percent, acid equivalent weight, total glucuronic acid, and total cationic composition were determined. The average values of moisture and ash percent of the samples were found to be 9.30% and 2.61% respectively, while the average specific rotation value of the samples was found to be(+49.40 ^{o}) .The determined intrinsic viscosity average value was found to be 11.40ml/g, while Kjeldahl determination of nitrogen content revealed an average of 0.14%. The calculated average protein values using a conversion factor 6.6 was 0.93%.The average values of acid equivalent weight and percentage of glucuronic acid were found to be 1107.9 and 15.9% respectively. It was found that Ca, Fe, K, Mg, Na and Cu were the major elements present in all samples. Three fractions of gum samples were obtained by solvent fractionation using absolute ethanol. The fractions were found to have varying amounts of protein and different values of intrinsic viscosity suggesting some variation in the molecular structure after fractionation.

1. Introduction

Gum Arabic along-chain, high-molecular weight polymer that dissolves in water to give a thickening effect is one of the oldest food ingredients. Despite periodical attempts to replace it [1], gum Arabic is still used widely in food industry due to it's texturing, film-forming, emulsifying, and stabilizing properties [2].

Acacia seyal var. seyal and Acacia senegal var. senegal are the two species of Acacia gums which constitute Gum Arabic [3]. Acacia seyal is less valued than Acacia Senegal due to its poor emulsification properties[4,5], therefore, it is considered to be an inferior quality gum. Commercial gum Arabic may contain up to ca 1% A.seyal as a contaminant .

The Joint Expert Committee on Food additives(JECFA) of FAO defined Gum Arabic as a dried exudation obtained from the stems and branches of Acacia senegal(L.) Willdenow or Acacia seyal(fam. Leguminosae)[6]. There are more than 1000 species of Acacia, but, the gum from Acacia senegal is, perhaps, the most valuable and widely used species of natural plant gums [7].

Ross [8] provided a comprehensive taxonomic study of the genus Acacia, which is one of the most widespread in Africa, he considered that the ancestral of Acacia evolved in central America, and that the subgenera Acacia and Aculeiferum differentiated in the same area.

Gum Arabic is a complex mixture of polysaccharides, protein and arabinoglacto protein(AGP) species. It has been shown to be highly heterogeneous and is found in nature as mixed calcium, magnisum, potassium and sodium salts of a polysaccharic acid (arabic acid). However, other heavy elements such as Zn, Al, Cd, Cu, Cr, Pb, and Co may also be present but in very small quantities [9,10,11].

Underwood et al [3] fractionated Gum Talha into eight fractions, with different protein contents, using Hydrophoic column chromatography. They[3] also used Ion-exchange chromatography, Solvent extraction and Ammonium sulphate as other means of fractionation.

Anderson et al [12] pointed out that the major analytical differences between A. senegal and A. seyal involve their nitrogen contents(0.33 % and 0.10 % respectively), rhamnose

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contents(12-14 % and 2-4 % respectively) and galactose/arabinose ratios (45:25 and 33:51 respectively), in addition to the differences between their specific rotaions(- 30 ^{o} ± 3o^{o} for A. Senegal; +50 ^{o} to +60 ^{o} for A.seyal). Moreover, Menzies et al [13] reported that A. senegal gums interact with β-glucosyl Yariv reagent, which is typical behavior for AGPs while A. seyal gums show either a very weak interaction or no interaction at all .

Table 1: Amino acid composition of some Gum Arabic samples (residues per 1000 residues)

Amino acid	a	B	C	
			1	2
Alanin	31	19	28	38
Argenine	7	0.0	15	11
Aspartic acid	60	39	91	65
Cystine	1.0	N.D	3.0	N.D
Glutamic acid	36	29	36	38
Glysine	49	69	53	51
Histidine	51	58	52	51
Hydroxyproline	274	327	256	240
Isoleucine	14	12	11	16
Leucine	75	68	70	85
Lysine	26	27	27	18
Methionine	N.D	N.D	2	N.D
Phenylalanine	29	N.D	30	24
Proline	72	73	64	73
Serine	123	163	144	170
Thereonine	72	70	72	62
Tyrosine	11	N.D	13	13
Valine	45	23	35	42

N.D ; not determined

a ; Average of 8 A. senegal samples.

b ; one A. senegal sample[11].

c; one sample 1 = A. senegal. 2 = A. seyal[10].

Anderson et al [9], hydrolyzed commercial Gum Arabic samples in 6 M hydrochloric acid and the hydrolysates were subjected to chromatographic analysis. Table 1 shows the amino acid composition of some gum Arabic samples. As shown in the table hydroxyproline and serine are the most abundant amino acids.

Smith degradation procedures on Acacia senegal showed that its a highly branched polysaccharide consisting of a main chain of β -1,3 - linked galactose residue with 1,6 – linked ramified side chains containing galactose, arabinose, rhamnose and glucouronic acids[9,10].

There are many publications concerned with the determination of average molecular mass of Gum Arabic [10,15,16].

Vandevelde et al [17], showed that Gum Arabic contains a high molecular mass protein rich fraction and a lower molecular mass protein deficient fraction. He described the high molecular mass fraction as an arabinogalactan protein complex(AGP).

Veis et al [18], reported weight average molecular mass value of $1.00X10^{6}$ using light scattering technique, while the values obtained by Vandevelde et al [17], range from $2.20X10^{5}$ to $1.00X10^{6}$ for Acacia senegal. However, Neil et al [19], fractionated Acacia senegal using hydrophobic interaction chromatography (HIC) into two fractions, hydrophilic (fraction 1, yield ≈ 80%) and hydrophobic (fraction 2, yield ≈ 2%).

Connoly et al [20], described the structure of Gum Arabic in terms of "Wattle Blossom Model" which includes the proteinaceous component and shows a number of arabinogalactan blocks of molecular mass ≈ $2.00 X 10^{5}$ linked to a polypeptide chain.

Johan et al [21], compared the molecular and the emulsifying properties of Gum Arabic and mesquite gum, while Shao-Ping et al [22], suggested an amendment to the classical core structure of Gum Arabic.

Gum Arabic has been used in a wide range of products for many years. Uses of Gum Arabic fall in three categories, Food, Pharmaceutical and Technical [23,24]. However, properties such as high solubility associated with low viscosity and absence of taste and odor made it easy to incorporate Gum Arabic in different food stuffs without disturbing their organo-leptic properties [25,26,27]. Gum is used in diary products such as ice creams, packed milk and processed baby foods. It is also used in the backing industry for its comparatively low water absorption properties and its favorable adhesive properties in glazes and toppings. One of the main uses of spray dried Gum Arabic is in solution form such as in beverages, beverage emulsion, and flavour emulsions such as orange juice , lemon juice , cherry and cola[28,29].

2. Materials and methods.

2.1 Materials.

Ten authentic samples of *Acacia seyal* var. *seyal* obtained from the Blue Nile region, Sudan, were provided by The Gum Arabic Company - Khartoum - Sudan.

2.2 methods

2.2.1 Purification of crude gum.

The gum samples used in this work were relatively pure, however, impurities such as wood pieces and sand particles were carefully removed by hand. Then each sample was

reduced to a fine powder using a mortar and pestle and kept in labeled self sealed polyethylene bags(0.04 mm thick).

2.2.2 Determination of moisture content.

Accurately 0.5 gram of each sample was weighed in a clean preheated and weighed dish. Then it was dried in an oven(WTC binder) at 105 ^{o}C for 12 hours to a constant weight. Moisture content was then calculated as a percentage of the initial weight.

2.2.3 Determination of ash.

Accurately 3 grams of the dried sample was ignited in a muffle furnace at 550 ^{o}C for 12 hours and ash % was calculated.

2.2.4 Determination of specific rotation.

A polarimeter(Leybold Didactic- GMBH 47283) was switched on and left to stabilize for one hour. The instrument was set to zero using distilled water as a blank. Gum solutions were prepared, 1% w/v, from the dry samples using distilled water, the gum solutions were first filtered through Watmann No. 42 filter paper. Then loaded into the sample holder without trapping air bubbles. Then the sample holder was placed in the instrument between the polarizing and analyzing prisms and the degree of rotation was measured and the corresponding specific rotation was then calculated. The cations were removed from the gum samples by passing 2 % gum solution through a column packed with Amberlite(IR-120 H^{+}) resin. The eluent was collected and evaporated to about half of its volume. Then 4.0 volumes of ethanol was added. The precipitated gum was separated, dried and its specific rotation was measured as described above.

2.2.5 Determination of intrinsic viscosity.

The gum solutions were prepared by dissolving 1 gram , of each sample, in 100 ml, 1 M, sodium chloride. The solutions were then filtered through Whatmann No. 40 filter paper. Five ml of the filtrate were then transferred into an Ostwald viscometer contained in water path set at 25 ^{o}C and left for half an hour. The efflux time was then measured for the original solution and two of its dilutions. The average of three measurements was taken for each concentration. The efflux time of the solvent, 1 M, sodium chloride was then measured and the intrinsic viscosity, for each sample, was calculated.

The intrinsic viscosity of the cations free gum solutions was also calculated as given above.

2.2.6 Determination of total nitrogen.

Kjeldahl method was used to determine the total nitrogen.

Exactly 0.5 gram of each sample, in duplicate, was weighed and transferred to Kjeldahl digestion tube plus one Kjeldahl tablet, copper sulfate-potassium sulfate catalyst. Then 10 mls of concentrated, nitrogen free, sulfuric acid were added. The tube was then mounted in the digestion heating system which was previously set to 240 ^{o}C and capped with an aerated manifold.

The solution was then heated at the above temperature until a clear pale yellowish-green color was observed which indicates the completion of the digestion . The tubes were then allowed to cool to room temperature. Their content was quantitatively transferred to Kjeldahl distillation apparatus followed by addition of distilled water and 30 %(w/v) sodium hydroxide. Steam distillation was then started and the released ammonia was absorbed in 25 ml of 2 % boric acid . Back titration of the generated borate was then carried out versus, 0.02 M, hydrochloric acid using methyl red as an indicator. Blank titration was carried in the same way .The percentage of nitrogen content was then calculated.

2.2.7 Determination of total glucouronic acid.

A cation exchange column packed with Amberlite(I R- 120 H^{+}) resin was first washed thoroughly with,2 M, sulfuric acid, then with distilled water until it is free from sulfates. Then 50 ml of 3 %(w/v) sample solutions, in duplicate, were slowly passed down the column. The eluent and washing, ca. 300 ml were collected and titrated versus standard,0.1 M, sodium hydroxide using phenolphthalein indicator [7]. Acid equivalent weight and percent uronic acid were then calculated.

2.2.8 Determination of cationic composition.

Atomic absorption measurements were made for the gum samples using Perkin Elmer 2280 atomic absorption spectrometer equipped with a deuterium arc background correction . The elements were determined using conventional hollow cathode lamps. The operating parameters were those recommended by the instrument manufacturer. The burner position, flame conditions and aspiration rate, were optimized for maximum absorbance.

2.2.9 Fractionation of Gum Arabic.

Solutions of 25% (w/v) gum acacia were prepared by hydrating the appropriate amount of gum overnight in distilled water. The gum solution was filtered through glass sintered funnel to remove any solid impurities. Then absolute ethanol, 99% BDH chemicals LTD Poole England, was added drop wise from a burette with continuous stirring until fraction(I) was separated. Fraction(I) was removed by decantation, dried at 60 ^{o}C over night, weighed and kept in a self sealed polyethelene bag for further analysis. After separation of fraction(I) ethanol was added to the same solution to separate fraction(II) which was treated as described in fraction(I). The same procedure was used to separate fraction (III) and fraction (IV) .

2.2.10 Determination of absorbance.

The absorbance of 1% solution of the obtained fraction was determined at 218 nm using UV-visible porotable datalogging spectrophotometer(HACH, DR, 2010).

3. Results and Discussion.

A number of physicochemical and chemical methods were used to characterize samples of *A. seyal var. seyal*, gums. Table 2 shows the percentage moisture and ash content of samples, the table shows that moisture content the samples fall between 5.66 % and 11.11%, while Ash percentage fall between 2.28%, and 2.93%. FAO food and nutrition paper 52[6], specifies that the loss on drying and total ash percentage as a purity test for Gum Arabic should not exceed 15% and 4% respectively.

Table 2: Moisture and ash. (%)

Sample number	Moisture(%)	Ash (%)
1	05.66	2.93
2	11.11	2.98
3	11.11	2.62
4	10.17	2.64
5	08.92	2.63
6	08.92	2.60
7	07.58	2.53
8	09.09	2.28
9	10.29	2.31
10	10.91	2.62
Mean	09.30	2.61
S.D	1.17	0.21
C.V	12.58	8.06

The average values of moisture and ash content and the appearance of the samples and the results shown in Table 2 fully agree with the above specification and description. Moreover, it agrees with the results of other workers[3,4, 12].

The specific rotation and intrinsic viscosity of the gum samples are shown in Table 3.

This table shows that *A. seyal* samples are dextrorotatory having specific rotation values falling between(+40^o) and (+54^o), while the intrinsic viscosity of the samples studied fall between 10.70 ml/g and 11.99 ml/g. The results obtained for all samples agree with the values cited in the literature [3,4,12].

Table 3 : Specific rotation and intrinsic viscosity

Sample number	Specific rotation (deg)	Intrinsic viscosity(ml/g)
1	+ 40	11.63
2	+ 46	11.63
3	+ 52	11.27
4	+ 46	11.73
5	+ 48	10.76
6	+ 54	10.70
7	+ 54	11.83
8	+ 52	11.27
9	+ 52	11.99
10	+ 50	11.42
Mean	+ 49.40	11.40
S.D	4.42	0.40
C.V	8.95	3.77

The small values of the standard deviation shown in Table 3 shows that the samples do not differ in their specific rotation and intrinsic viscosity values. However, a slight insignificant change was noted in the specific rotation and intrinsic viscosity values of the deionised gum solutions. The change was a decrease, in the range of 1 to 3 units, in the intrinsic viscosity values and an increase, in the range of 2 to 5 units, in the specific rotation values this is shown in Figures1 and 2 which compare the values of the intrinsic viscosity and specific rotation obtained before and after removal of cations of 1107.9 and 15.9 respectively. Acid equivalent weight and the calculated glucouronic acid values are in a good agreement with the values reported in the literature .

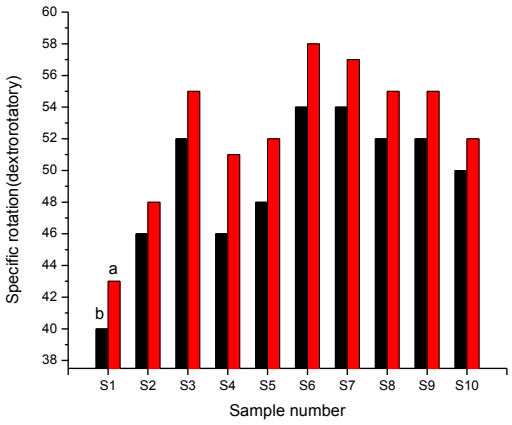

Figure 1: Specific rotation before(b) and after(a) removal of cations

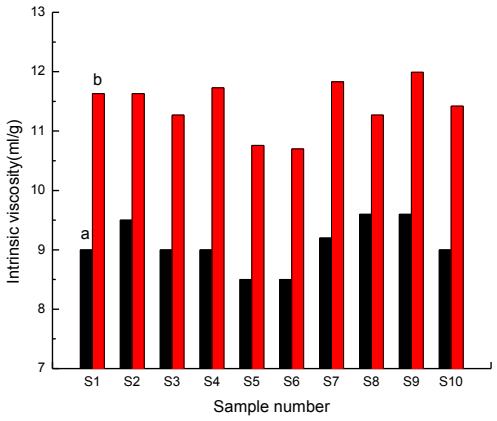

Figure 2: Intrinsic viscosity before(b) and after(a) removal of cations

Nitrogen and protein content calculated using nitrogen conversion factor 6.6[6] are shown in Table 4, the table shows that the average nitrogen and protein percent of samples are 0.14 % and 0.931 % respectively. Nitrogen and protein values which are shown in Table 3 are in good agreement with the specification for identity and purity of Gum Arabic of FAO and with the values obtained by other workers [3,4,].

Table 4 : Nitrogen and protein content (%)

Sample number	Nitrogen(%)	Protein(%)
1	0.12	0.792
2	0.15	0.990
3	0.18	0.792
4	0.12	1.188
5	0.13	0.858
6	0.14	0.942
7	0.14	0.942
8	0.17	1.122
9	0.13	0.858
10	0.13	0.858
Mean	0.14	0.931
S.D	0.02	0.140
C.V	14.18	15.04

The acid equivalent weight and the corresponding calculated glucouronic acid contents are given in Table 5. The Table shows that the acid equivalent weight of the samples fall between 980 and 1179 with the corresponding glucouronic acid within the range of 15% and 18% with an average values

FAO food and nutrition paper 52[6] includes in its definition of Gum Arabic that it consists mainly of high molecular weight polysaccharides and their calcium, magnesium and potassium salts.

Table 5 : Acid equivalent weight and glucouronic acid(%).

Sample number	Acid equi.wei.	Glucouronic acid(%)
1	0980	18.0
2	1128	15.6
3	1153	15.3
4	1081	16.3
5	1128	15.6
6	1045	16.9
7	1104	16.0
8	1128	15.6
9	1179	15.0
10	1153	15.3
Mean	1107.9	15.9
S.D	59.10	0.92
C.V	5.33	5.72

Table 6 shows that calcium, magnesium, potassium, sodium, iron, and copper are the most abundant elements in all seyal gum samples. The mean values in the table show that the major elements in *A. seyal var. seyal* samples are, in the increasing order Ca, Fe, K, Mg, Na, and Cu. The high standard deviation values of these elements indicate that there is a considerable variation in their amounts from one sample to another. However, it is evident that the elemental composition is the main reason which contributes to color and appearance of different nodules of the same variety or even within different regions in the same nodule.

Moreover, Fe and Cu are transition metals which form colored complexes, therefore they are considered to be the main reason for coloration. However, most elements are soil dependent, therefore their amounts are expected to increase or decrease within different regions in the same area according to the soil type. Other element namely Zn , Co , Al , Ni , Cd , As , Cr and Mo were not detected in the given samples, this agrees with what was reported on our previous work on gum Arabic samples[33]

Table 6 : cationic composition (ppm)

Sample number	Ca	Mg	Na	K	Fe	Cu
1	10000	1230	750	2416	1785	20.0
2	9800	650	878	2416	3392	25.7
3	10000	650	666	3583	3392	18.6
4	9615	650	N.D	2916	8035	15.7
5	10000	823	250	2500	5000	22.8
6	10000	500	250	2500	4642	20.0
7	10750	883	250	2500	4107	15.7
8	8333	1000	250	2416	4612	17.0
9	9487	475	750	3083	4821	17.0
10	10250	750	N.D	2500	3571	15.7
Mean	9824	761	505.50	2683	4339	18.82
S.D.	554.95	231.61	279.08	390.46	1614.9	3.36
C.V.	5.65	30.43	55.21	14.55	37.22	17.85

Table 7 : Physicochemical data for A. seyal fractions.

Frac. num.	yield (%)	Nitrogen (%)	Protein (%)	Spe.rot (deg.)	Intr.vis (ml/g)	Abs. at 218 nm
I	36.4	0.37	2.44	+ 60	10.95	0.528
II	5.2	0.42	2.77	+ 60	12.30	0.055
III	11.6	0.82	5.41	+ 80	11.12	0.045
IV	14.4	0.82	5.41	+ 120	10.24	0.032

The data given in Table 7 shows that four fractions were obtained for *A. seyal var. seyal* . The Table also shows that fractions,(III) and (IV) do not differ in their nitrogen and protein contents. The protein contents of fractions(I), (II), (III) and (IV) were 2.44 %, 2.77 %, 5.41 and 5.41%, respectively. Moreover, the major proportion of the proteinaceous material is confined to fractions(III) and (IV). The results also show that the majority of the material was contained in fraction (I), this is supported by the absorbance values shown. As shown in the table fraction(I) is the lowest in protein while fractions (III) and (IV) are the richest in protein. This agrees, to a great extent, with values reported[3,4,5,12].

The specific rotation values obtained for the fractions are also shown in Table 7. The Table shows that the intrinsic viscosity values for fractions (I), (II), (III) and (IV) were 10.95,12.30, 11.12 and 10.24ml/g respectively. The results also point out that the intrinsic viscosity of fraction(II) is higher than that of the whole gum (11.40ml/g). The results agree with what was reported by other workers .

The specific rotation values were found to change suggesting an alternation in the chemical structure of the polysaccharide component after fractionation. However, the reported results show values of($+60^o$) for fraction(I) and(II). While the values were($+80^o$) and ($+120^o$) for fractions(III) and(IV) respectively. Nonetheless, the values shown agree with what was reported by other means of fractionation [9].

4. Conclusion;

From this study the following can be concluded ;

1-A. seyal var. seyal samples studied meet some of the specifications given by The Joint Expert Committee for Food Additives for Gum Arabic

2- There is no significant natural variability among A. seyal var. seyal samples despite the differences in appearance and color.

3- A. seyal var. seyal gums can be fractionated using ethanol to three fractions with varying concentrations of protein, different values of specific rotation and intrinsic viscosity .

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