Bull. Chem. Soc. Ethiop. **2003**, 17(1), 67-74. ISSN 1011-3924
Printed in Ethiopia

INTRA-SPECIES VARIATION OF THE PROPERTIES OF GUM EXUDATES FROM *ACACIA SENEGAL* VAR. *SENEGAL* AND *ACACIA SEYAL* VAR. *FISTULA* FROM TANZANIA

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(Received May 22, 2002; revised November 13, 2002)

ABSTRACT. Gum exudates from *Acacia senegal* var. *senegal* and *Acacia seyal* var. *fistula* from Tanzania have been analyzed and their inter- and intra-species variation of their properties evaluated. The results show that significant inter-species variation of the properties of the gum exudates from the two species exist, whereas only some parameters show significant intra-species variation. The specific optical rotations of the gum exudates have been found to vary from −43.2° to −52° for *Acacia senegal* var. *senegal* and +36.0° to +60.0° for *A. seyal* var. *fistula.* Likewise, the acid equivalent weights (AEWs) have been found to vary from 1093 to 1920 for *A. senegal* var. *senegal* and 1063 to 1749 for *A. seyal* var. *fistula*. Significant intra-species variation for the tannin content has also been observed for the species studied, whereas the nitrogen content has been observed to vary significantly only in *A. seyal* var. *fistula.* The results in this study show that the variation of the properties among batches of commercial *Acacia* gum may be due to admixture of gums from different *Acacia* species as well as intra-species variation.

KEY WORDS: *Acacia senegal* var. *senegal*, *Acacia seyal* var. *fistula*, Gum exudates, Intra-species variation, Inter-species variation

INTRODUCTION

About 500 species of *Acacia* are distributed throughout the Sahelian regions of Africa, however, it is recognized that the main source of commercial *Acacia* gum is *Acacia senegal*. We have previously reported [1, 2] that commercial *Acacia* gum from Tanzania is obtained mainly from *A. drepanolobium* and gum exudates from other *Acacia* species of the series Gummiferae may find their way into the commercial shipments. Systematic studies in our laboratory have shown that some properties of the gum exudates from *A. kirkii* ssp.*kirkii* var. *kirkii* and *A. malacocephala* resemble those of Tanzanian commercial *Acacia* gum. In general, the variation of the properties of commercial *Acacia* gum (gum arabic) has mainly been attributed to adulteration by other *Acacia* species. Our previous work [3] has also shown that variants of A. *senegal* e.g. A *senegal* var. *leiorhachis* which may also find their way into commercial gum arabic shipments may have solution properties which vary significantly from the main species. This paper presents the intra-species variation of the properties of gum exudates from *Acacia senegal* var. *senegal* and *A. seyal* var. *fistula* and will presumably contribute in underpinning the reasons for the variation of the properties of commercial *Acacia* gum.

RESULTS AND DISCUSSION

The physicochemical data for the samples studied are summarized in Tables 1-4. Cold water insoluble gel (CWIG) and hot water insoluble gel (HWIG) are the proportions of the gum that

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do not dissolve in cold and hot water, respectively, even after prolonged contact with water. The insoluble matter (bark, twigs, sand, etc.) are not very important because they can largely be minimized by hand grading. Whereas all the *Acacia* gum exudates from *A. senegal* var. *senegal* (range between 1.23-2.08% for CWIG and 0.77-1.84% w/w for HWIG) investigated possess low CWIG and HWIG contents, for *A. seyal* var. *fistula* gum, samples collected from different geographical locations have shown to vary significantly in their CWIG and HWIG contents. Thus, the difference between the lowest and the largest values (0.52-7.91% w/w for CWIG and 0.41-6.70% w/w for HWIG) is quite large. High values of CWIG and HWIG have also been reported for gum exudates from *A. malacocephala* (CWIG, 5.88; HWIG, 3.92 % w/w) [2], *A. polyacantha* ssp. *campylacantha* (CWIG, 36.70; HWIG, 10.90% w/w) and *A. tortilis* ssp. *spirocarpa* (CWIG, 13.30; HWIG, 11.60% w/w) [3].

The data in Table 1 shows that the viscosities of four gum samples from *A. senegal* var. *senegal* are essentially similar. However, one of the samples (AS V) recorded a viscosity which is twice the average of the other four samples. It has been suggested [4] that there is a direct relationship between the amount of insoluble gel and the viscosity of the resultant gum solution. Jefferies *et al.* [5] has shown that the insoluble fraction of commercial gum ghatti varies from 8-23% and the viscosity of the gum depends on the proportion of the insoluble fraction, meaning that there is a wide variation of viscosity from batch to batch. However, this effect has not been established for *Acacia* gums. The origin of sample AS V is the same as that of samples AS I-III thereby ruling out the possibility of difference in geographical location, and the proportion of its insoluble gel is also not significantly different from the three samples. The reason for the high viscosity of this sample is, therefore, unclear. For the *A. seyal* var. *fistula* gum samples the viscosities of all the five samples studied are similar. They are also similar to the values obtained previously for Tanzanian commercial *Acacia* gums [1, 2].

Table 1. Physicochemical properties of *Acacia senegal* var. *senegal* and *Acacia seyal* var. *fistula* gums.

Parameter	*A. senegal* var *senegal*					*A. seyal* var. *fistula*				
	AS I	AS II	AS III	AS IV	AS V	AF I	AF II	AF III	AF IV	AF V
Moisture % w/w	13.20	13.59	14.76	15.63	15.05	12.45	13.19	14.08	14.68	14.68
Ash % w/w	3.81	3.38	3.36	4.56	4.46	2.84	2.86	2.74	2.75	2.29
Acid insoluble matter % w/w	0.92	0.99	0.81	0.28	0.88	0.54	0.27	0.84	1.95	2.16
CWIG % w/w	1.23	1.75	2.08	1.15	1.57	1.08	0.52	3.23	6.64	7.91
HWIG % w/w	1.15	1.19	1.84	0.77	0.96	0.74	0.41	2.27	5.67	6.70
Methoxyl %w/w	0.16	0.19	0.15	0.16	0.17	1.07	1.20	1.11	1.13	1.18
Nitrogen % w/w	0.33	0.32	0.32	0.37	0.33	0.26	0.25	0.28	1.14	1.42
$[\alpha]_D$ in H_2O, deg.	-43.2	-44.0	-52.0	-47.6	-49.0	+52.0	+55.0	+36.0	+60.0	+60.0
Viscosity (cP)										
100 gL^{-1}	2.10	2.56	2.30	1.69	4.61	5.65	4.21	3.73	4.37	3.36
150 gL^{-1}	4.72	4.68	5.12	3.02	9.44	14.42	10.89	10.85	7.78	8.15
Optical density (ABS)	0.12	0.14	0.12	0.06	0.06	0.30	0.14	0.40	0.55	0.37
Tannin % w/w	0.54	0.58	0.48	0.35	0.28	0.85	0.58	0.87	1.19	0.76
Acid equivalent weight	1094	1452	1920	1340	1576	1510	1749	1136	1119	1063

Key: AS I-V = *Acacia senegal* var. *senegal* gum samples; AF I-V = *Acacia seyal* var. *fistula* gum samples.

Table 2. Range and average of the properties of *A. senegal* var. *senegal* and *A. seyal* var. *fistula* gums.

Parameter	*A. senegal* var. *senegal* (5 samples)		*A. seyal* var. *fistula* (5 samples)	
	Range	Ave.	Range	Ave.
Moisture % w/w	13.2-15.6	14.4	12.5-14.7	13.8
Ash % w/w	3.4-4.6	3.91	2.3-2.9	2.70
Acid insoluble matter % w/w	0.3-1.0	0.78	0.3-2.2	1.15
CWIG % w/w	1.2-2.1	1.56	0.5-7.9	3.88
HWIG % w/w	0.8-1.8	1.18	0.4-6.7	3.16
Methoxyl % w/w	0.15-0.19	0.17	1.1-1.2	1.14
Nitrogen % w/w	0.32-0.37	0.33	0.25-1.42	0.67
$[\alpha]_D$ in H_2O, deg.	(-43)-(-52)	-47	(+36)-(+60)	+53
Viscosity (cP) 100 gL^{-1}	1.7-4.6	2.65	3.4-5.7	4.26
Optical density (ABS)	0.06-0.14	0.10	0.14-0.55	0.35
Tannin % w/w	0.3-0.6	0.45	0.6-1.2	0.85
Acid equivalent weight	1094-1920	1476	1063-1749	1315

Acacia gums are arabinogalactan proteins and nitrogen occurs in the form of amino groups ($-NH_2$) on the terminal amino acid residue of the protein and the peptide linkages (-NHCO-). Nitrogen content is considered as one of the very useful parameters in distinguishing gums from different species [6, 7]. For *A. senegal* var. *senegal* all the five samples studied show almost the same value of nitrogen content. The gum samples from *A. seyal* var. *fistula,* however, show significant intra-species variation. Two samples, AF IV and AF V which were obtained from Mnang'ana village, 18 km west of Singida, have shown much higher values (1.14% and 1.42% w/w, respectively, *i.e.* almost five times) of nitrogen content than the average of the three samples (0.26% w/w) from Kwamtoro and Mnzakwe villages. A significant variation in the properties of gum exudates obtained from the same species in different geographical locations has also been observed previously [6]. Values of nitrogen content which are similar to or even higher than samples AF IV and AF V have also been reported previously for *A. drepanolobium* gum (1.11-1.12% w/w) [8], *A. tortilis* ssp *spirocarpa* gum (2.54% w/w) [3] and commercial *Acacia* gum from Kenya [9].

Among the analytical parameters, specific rotation shows remarkable distinction between the series Gummiferae and Vulgares. Members of the former are known to exude dextrorotatory gums (+51° to +108°) whilst members of the latter exude laevorotatory gums (-12° to –30°) [6]. Exceptions to this observation include *A. macracantha* gum with optical rotation -6° to -18° [10], *A. tortousa* with optical rotation -25° to -29° [11] and *A. ehrenbergiana* with optical rotation -3° to -9° [12] although these gums are from the series Gummiferae. *A. senegal* var. *senegal* belongs to the series Vulgares whereas *A. seyal* var. *fistula* belongs to the series Gummiferae. *Acacia* gum to be used for food and pharmaceutical applications should be leavorotatory. In this work, the specific optical rotations of all the *A. senegal* var. *senegal* gum samples (-43.2° to -52°) were found to be leavorotatory. The values are also within the range expected for gums from *A. senegal* var. *senegal* [2, 9, 13]. For the *A. seyal* var. *fistula* gum samples, the intra-species variation of the specific optical rotation is more pronounced. One of the samples obtained from Mnzakwe village has shown a remarkably lower value of specific rotation (+36°) as compared to the rest of the samples (Table 1). A similar observation has been reported previously by Duvalett *et al.* [13] who recorded a wide range of optical rotations (-25° to –62°) for 75 samples of *Acacia senegal* gum suggesting that this is probably due to variation among the exuding trees.

The methoxyl content of *Acacia* gums varies from species to species. Gums from members of the series Gummiferae contain [6, 14] methoxyl content at generally higher levels (0.47-2.4% w/w) than those from the series Vulgares (0.25-0.75% w/w). From the results, a significant variation of the methoxyl content is observed between the two species, whereas no significant intra-species variation is observed regardless of the place of origin. This suggests that there are variations in the abundance of 4-O-methylglucuronic acid moieties among species of the same genera, implying that the chemical structures of gum exudates from different species are quite different. The values obtained in this work for the gum exudates from *A. seyal* var. *fistula* (average 1.14% w/w) are similar to that reported previously for *Acacia seyal* (0.94% w/w) [15] and within the range expected for gums from *Acacia* species of the series Gummiferae. Those of *A. senegal* var. *senegal* gum samples are similar to that reported by Mrosso [2].

Some *Acacia* species are widely grown in Africa for the commercial production of tannin which is extracted from the bark. These are, by their origin, the Australian *Acacia* species belonging to the series Botryocephalae. Some *Acacia* species of the series Gummiferae and Vulgares are also known to produce tannin but in smaller amounts. It is believed that the tannin content of gums increases as the gum ages on the tree. The tannin content of *A. senegal* var. *senegal* gum, in this study, was shown to vary from 0.28 to 0.58% w/w. The gum exudate from *Acacia senegal* var. *leiorhachis,* a close relative of *A. senegal* var. *senegal* from Tanzania, has also been shown to contain tannin (0.37% w/w) [3]. Previous analysis of fifty seven gum samples of *A. senegal* var. *kerensis* by Chikamai and Banks [9] showed the gum to contain no tannin. The values obtained in this work for *A. senegal* var. *senegal* gum are similar to that reported previously for a gum sample from the same species (0.28% w/w) [2], *Acacia polyacantha* ssp *campylacantha,* 0.58% w/w and *Acacia tortilis* ssp *spirocarpa*, 0.50% w/w) from Tanzania [3]. Anderson and Morrison [15] reported the tannin content of some *Acacia* gum exudates from the series Gummiferae as *A. fischeri,* 0.65% w/w; *A. kamarunensis,* 0.33% w/w*; A. spirocarpa,* 1.0% w/w; *A. stenocarpa,* 0.85% w/w and *A. seyal,* 1.9% w/w. The reference sample of *A. senegal* gum (the sample used as the representative test article in toxicological studies to establish the safety of gum arabic as a food additive) was found to contain no tannin [16].

Potentiometric titration furnished the natural pH, percentage salt form of anionic groups and acid equivalent weight of the gums. The analyzed samples are slightly acidic in water as shown by their natural pH values, which range from 4.37 to 4.95 and are in good agreement with the pH values reported previously [9] for *Acacia* gums. The acid equivalent weight (AEW) of a gum is defined as the mass of the gum that contains one equivalent of uronic acid. A high value of AEW indicates a low uronic acid content in the gum and *vice versa.* The data in Table 1 show that there is a wide intra-species variation for the acid equivalent weights (range 1094-1920) of the *A. senegal* var. *senegal* gum samples. Two samples, one from Mbande village, 63 km from Dodoma on the Dodoma to Morogoro road and the other from Chalinze village, 67 km east of Dodoma have recorded low values (1094 and 1340) of AEW. It is interesting to note that the other three samples from Mbande village recorded different AEW suggesting that the variation of the AEW cannot be ascribed to geographical location. A wide range of AEW for *A. seyal* var. *fistula* gum samples (1063-1749) is also observed, indicating a wide intra-species variation. Studies to find out whether these differences may be ascribed to the age of the plants are in progress.

The maximum limit for total ash for food and pharmaceutical quality *Acacia* gum is 4.0% w/w. Whereas all the gum samples from *A. seyal* var. *fistula* studied have shown to meet this specification, two samples of *A. senegal* var. *senegal* have shown to exceed the limit. Table 3 shows the metal composition of the studied gum samples. Previous reports [9, 15] have shown that the abundance of alkali and alkaline earth metals in *Acacia* gum exudates follow the trend Ca > K > Mg > Na. The results obtained in this work, however, shows that four of the gum

samples from *A. senegal* var. *senegal* have potassium as the most abundant metal. A similar observation has previously been reported by Mhinzi and Mrosso [3] for gums from *Acacia polyacantha* ssp. *campylacantha* and *Acacia senegal* var. *leiorhachis*. The values obtained for *A. seyal* var. *fistula* gum samples, however, are consistent with the literature values in that calcium is the most abundant metal followed by potassium, magnesium and sodium. Calcium ions are known to be responsible for gel formation in some tree exudate gums such as *Khaya grandifoliola* [17, 18] but this effect has not been reported for *Acacia* gums. The level of trace elements in *Acacia* gums has been reported [9, 15] to follow the trend iron > copper > zinc > lead. This work shows higher levels of zinc than copper for all the *A. senegal* var. s*enegal* gum samples. It is interesting to note also that three *A. seyal* var. *fistula* gum samples have recorded levels of zinc which are higher than iron.

Table 3. Cation content of *Acacia senegal* var. *senegal* and *Acacia seyal* var. *fistula* gums.

Sample	Metal content												
	% w/w				ppm								
	Na	K	Ca	Mg	Fe	Zn	Pb	Cu	Ni	Cd	Mn	Cr	Co
AS I	0.021	0.967	0.452	0.160	74.376	25.066	7.857	1.235	5.238	0.150	3.405	0.748	BDL
AS II	0.009	0.921	0.457	0.154	42.111	13.402	6.473	1.256	1.904	0.381	6.130	4.569	BDL
AS III	0.012	0.893	0.432	0.147	78.927	23.051	19.702	0.788	7.487	0.197	3.704	4.729	BDL
AS IV	0.004	0.626	0.989	0.042	18.500	13.087	14.500	1.371	0.000	0.000	2.273	0.392	BDL
AS V	0.014	0.984	0.592	0.290	98.000	19.450	4.290	6.670	nd	nd	nd	nd	nd
AF I	0.062	0.142	0.723	0.070	55.316	17.478	10.947	2.372	4.379	0.109	0.949	1.460	BDL
AF II	0.040	0.105	0.813	0.054	26.322	12.198	11.329	1.133	0.000	0.227	0.453	0.000	BDL
AF III	0.073	0.224	0.712	0.061	10.385	26.233	0.775	0.000	0.000	0.000	1.821	0.000	BDL
AF IV	0.065	0.149	0.743	0.079	9.103	16.178	11.474	0.000	1.474	0.076	11.053	0.755	BDL
AF V	0.011	0.082	0.249	0.067	12.467	24.534	7.192	2.000	1.998	0.000	5.714	0.000	BDL

Key: AS I-V = *Acacia senegal* var. *senegal* gum samples, AF I-V = *Acacia seyal* var. *fistula* gum samples, BDL = below detection limit, nd = not determined.

Table 4. Average level of some metals in *Acacia senegal* var. *senegal* and *Acacia seyal* var. *fistula* gum samples.

Metal	*A. senegal* var. *senegal* (5 samples)	*A. seyal* var. *fistula* (5 samples)
Na % w/w	0.011	0.050
K % w/w	0.878	0.140
Ca % w/w	0.584	0.648
Mg % w/w	0.159	0.066
Fe ppm	62.38	22.12
Zn ppm	18.81	19.32
Pb ppm	10.56	8.34
Cu ppm	2.26	1.10
Ni ppm	3.66	1.57
Cd ppm	0.182	0.082
Mn ppm	3.88	4.00
Cr ppm	2.61	0.443

The maximum permissible lead content in *Acacia* gum for food and pharmaceutical applications has been specified by the Joint Expert Committee for Food Additives (JECFA) [19]

of FAO as not more than 2 ppm. In this work all the gum samples, except sample AF III from *A. seyal* var. *fistula* gum (see Table 3) have been found to contain higher than the permissible levels of lead. Metal ion composition is a function of the soil on which the plants grow and may therefore vary within samples obtained from the same species [9]. The high levels of lead in these samples may be attributed to high levels of lead in the respective soils.

This work has shown that some physicochemical parameters of gum exudates from *A. senegal* var. s*enegal* and *A. seyal* var. *fistula* from Tanzania show significant inter- and intra-species variation. The inter-species variations are well documented and implies that the molecular structures of gum exudates from different species may be different. The intra-species variations are presumably resulting from difference in geographical location as has been proposed by Kapoor *et al,* [20] or age of plant [21]. However, Duvallet *et al.* [13] reported differences in intrinsic viscosities (between 0.14 and 0.60 decilitre/gram), specific optical rotation (between -25˚ to -62˚) and nitrogen content (between 0.12 and 0.57%) in gum exudates from plants of the same species obtained from the same experimental field and the gums not subjected to mechanical or thermal effects. It was also observed that the viscosities and optical rotations did not vary significantly after storage for one year in the laboratory. The variation of the properties among batches of commercial *Acacia* gum may therefore be due to admixture of gums from different *Acacia* species as well as intra-species variation.

EXPERIMENTAL

Origin of samples

Five gum samples from each of the *Acacia* species were collected from central Tanzania in the commercial *Acacia* gum producing areas as follows:

Acacia senegal var. *senegal* gum samples:

	Place of origin
AS I	Mbande village, 63.0 km from Dodoma on the Dodoma to Morogoro road.
AS II	Mbande village, 63.0 km from Dodoma on the Dodoma to Morogoro road.
AS III	Mbande village, 63.0 km from Dodoma on the Dodoma to Morogoro road.
AS IV	Near Ikowa Dam, Chalinze village, 67 km East of Dodoma (sample from a single tree).
AS V	Mbande village, 63.0 km from Dodoma on the Dodoma to Morogoro road.

Acacia seyal var. *fistula* gum samples:

	Place of origin
AF I	Kwamtoro village, 22 km from Dodoma on Dodoma - Singida (via Manyoni) road.
AF II	Mnzakwe village, 24 km from Dodoma on Dodoma - Arusha road.
AF III	Mnzakwe village, 24 km from Dodoma on Dodoma - Arusha road.
AF IV	Mnang'ana village, Singida district, 18 km West of Singida on Singida to Mlandara road.
AF V	Mnang'ana village, Singida district, 18 km West of Singida on Singida to Mlandara road.

Botanical vouchers from each of the species were also collected and confirmation of the species was obtained in the Herbarium, Botany Department, University of Dar es Salaam.

Procedures

Moisture content was determined by heating at 105 ^{o}C to constant weight. Ash and acid insoluble matter were determined by standard methods [22]. Cold water insoluble gel (CWIG) and hot water insoluble gel (HWIG) are the proportions of the gum (excluding twigs, sand, leaves, etc.) which are insoluble in cold and hot water, respectively, after prolonged stirring (2 hours). Methoxyl content was determined by the Zeisel's method whilst nitrogen content was determined by the Kjeldahl method. A Model AA-10 automatic polarimeter manufactured by Optical Activity Ltd., UK was used to measure specific rotations. The viscosities of 100 and 150 g L^{-1} aqueous solutions were determined using ubbleholde suspended level capillary viscometers. The tannin content was determined using tannic acid as a standard as described by Anderson and Morrison [15] whereas the acid equivalent weights were obtained by potentiometric titrimetry. Atomic absorption spectrophotometry (Perkin Elmer Model 2380 double beam instrument) was used for the determination of all the metals except sodium and potassium which were determined by the flame emission technique using the same instrument. All the determinations were carried out in duplicate and averaged (five separate readings were carried out for specific rotation). The detailed experimental procedures for all the parameters have been described previously [3, 23, 24].

ACKNOWLEDGEMENTS

The author wishes to thank NORAD for financial support and Mr. Frank Mbago of the Herbarium, Botany Department, University of Dar es Salaam for the identification of the *Acacia* species.

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