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Intra-species variation of the properties of gum exudates from two *Acacia* species of the series Gummiferae

G.S. Mhinzi *, L.A.R. Mghweno, J. Buchweishaija

Chemistry Department, University of Dar es Salaam, P.O. Box 35061, Dar es Salaam, Tanzania

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Abstract

Gum exudates from *Acacia drepanolobium* and *A. kirkii* ssp. *kirkii* var. *kirkii* from Tanzania have been analyzed and the intra-species variation of their properties evaluated. The results show that inter-species variation of the properties of the gum exudates from the two species exist, whereas only some parameters show intra-species variation. The specific optical rotations of the gum exudates varied from +72.0° to +94.6° for *A. drepanolobium* and +29.2° to +38.0°, for *A. kirkii* ssp. *kirkii* var. *kirkii.* Likewise, the acid equivalent weights (AEWs) varied from 832 to 1659 for *A. drepanolobium* and 663 to 1199 for *A. kirkii* ssp. *kirkii* var. *kirkii*. Intra-species variation for the tannin content was also observed for the species studied, whereas no intra-species variation was observed for the nitrogen and methoxyl contents. The results in this study show that the variation of the properties among the batches of commercial *Acacia* gum may be due to admixture of gums from different *Acacia* species, as well as intra-species variation.

Keywords: Gum exudates; *Acacia*; Properties; Gummiferae

1. Introduction

Acacia gum (gum arabic) is a natural polysaccharide hydrocolloid which is a permitted food additive and is defined (JECFA/FAO, 1999) as "the dried exudate obtained from the stems and branches of *Acacia senegal* (L) Willdenow or *Acacia seyal* (Family Leguminosae)". The gum is used in several applications, including dairy products, chewing gums, snack foods, gelatins and puddings. Work by Duvallet, Fenyo, and Vandevelde (1993) has shown that some physicochemical properties of the gum exudate from *A. senegal* may vary significantly, even among trees from the same geographical location. However, a report by Idris, Williams, and Phillips (1998) on eight authenticated samples showed that no obvious trends could be observed with respect to age or source of the tree for the monosaccharide composition, protein and amino acid contents and optical rotations for gum exudates obtained from *A. senegal* from two different areas in Sudan. In a continued investigation of the properties of authenticated *Acacia* gum exudates from Tanzania in our laboratory, we considered whether such consistency also exists, particularly in the major source of Tanzanian commercial *Acacia* gums, *Acacia drepanolobium.* Previous studies in our laboratory (Mrosso, 1996) have shown that some properties of the gum exudate from *A. drepanolobium* resemble those of Sudanese commercial *Acacia* gum. In general, the variation of the properties of commercial *Acacia* gum (gum arabic) has mainly been attributed to adulteration by gums from other *Acacia* species, as well as gums from *Combretum* and *Albizia* species. Recent work by Mhinzi (2003), however, has shown that some *Acacia* species from Tanzania produce gums which show intra-species variation in some of their physicochemical properties. This paper presents the intra-species variation of the properties of gum exudates from *A. drepanolobium* and *A. kirkii* ssp. *kirkii* var. *kirkii* (the two species belong to the series Gummiferae)

* Corresponding author. Tel.: +255 784 387268; fax: +255 22 2410038.
E-mail address: mhinzi@chem.udsm.ac.tz (G.S. Mhinzi).

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and will contribute in underpinning the reasons for the variation of the properties of commercial *Acacia* gum from Tanzania.

2. Materials and methods

2.1. Origin of samples

Five gum samples from each of the *Acacia* species were collected from central Tanzania in the commercial *Acacia* gum-producing areas (Scheme 1).

Botanical vouchers (branches, twigs, fruits, pods, seeds) of the respective trees were collected, along with the gum samples, and deposited in the herbarium (Botany Department) of the University of Dar es Salaam and the confirmation of the species identification was obtained from the Royal Botanic Gardens Herbarium, Kew, UK.

2.2. Experimental procedures

Moisture content was determined by heating at 105 °C to constant weight. Ash and acid-insoluble matter were determined by standard methods as detailed in the Food Chemicals Codex (1981). Cold water-insoluble gel (CWIG) and hot water-insoluble gel (HWIG) are the proportions of the gum (excluding twigs, sand, leaves), which are insoluble in cold and hot water, respectively, after prolonged stirring (2 h). Methoxyl content was determined by the Zeisel 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 (1989), whereas the acid equivalent weights were obtained by potentiometric titrimetry. Atomic absorption spectrophotometry (PerkinElmer 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 (Mhinzi & Mosha, 1993, 1995).

3. Results and discussion

The physicochemical data for the samples studied are summarized in Tables 1–4. The insoluble fraction of commercial *Acacia* gum is important because it is a measure of the quality of the gum, and good quality *Acacia* gum

Sample code	Place of origin
Acacia drepanolobium	
AD I	Near Kintinku village, 73.0 km from Dodoma on the Dodoma - Singida (via Manyoni) road.
AD II	Near Kintinku village, 73.0 km from Dodoma on the Dodoma - Singida (via Manyoni) road.
AD III	Near Kintinku village, 73.0 km from Dodoma on the Dodoma - Singida (via Manyoni) road.
AD IV	Kibigiri (near Shelui village), 84 km from Singida on Singida to Igunga road.
AD V	Kibigiri (near Shelui village), 84 km from Singida on Singida to Igunga road.
Acacia kirkii ssp. *kirkii* var. *kirkii*	
KK I	Msembeka village, 38 km from Dodoma on Dodoma - Singida (via Manyoni) road.
KK II	Msembeka village, 38 km from Dodoma on Dodoma - Singida (via Manyoni) road.
KK III	Msembeka village, 38 km from Dodoma on Dodoma - Singida (via Manyoni) road.
KK IV	Ipala - Ngayegae, on Dodoma - Morogoro road.
KK V	Chalinze village, 52 km from Dodoma on Dodoma to Morogoro road.

Scheme 1. Gum samples.

Table 1
Physicochemical properties of *Acacia drepanolobium* and *Acacia kirkii* ssp. *kirkii* var. *kirkii* gums

Parameter	*Acacia drepanolobium*					*Acacia kirkii* ssp. *kirkii* var. *kirkii*				
	AD I	AD II	AD III	AD IV	AD V	KK I	KK II	KK III	KK IV	KK V
Moisture (% w/w)	12.40	13.22	12.85	14.54	14.64	13.61	13.11	13.69	15.11	15.80
Acid-insoluble matter (% w/w)	0.25	0.30	0.33	0.78	0.67	0.20	0.12	0.20	0.11	0.82
CWIG (% w/w)	1.18	1.13	1.15	1.49	1.62	0.86	0.66	0.79	1.60	3.46
HWIG (% w/w)	0.78	0.81	0.61	1.01	1.10	0.75	0.52	0.67	1.50	1.94
Ash (% w/w)	2.38	2.44	2.40	2.25	2.24	4.26	3.89	3.85	4.50	4.42
Viscosity (cP)										
100 g l^{-1}	3.54	2.76	3.02	2.32	2.83	3.45	3.03	3.39	5.82	3.34
150 g l^{-1}	8.62	6.34	6.86	5.02	6.20	7.24	6.61	8.22	12.70	7.35
Methoxyl (% w/w)	0.84	0.87	0.92	0.87	0.90	0.45	0.43	0.39	0.47	0.46
Nitrogen (% w/w)	0.49	0.49	0.49	0.49	0.48	0.33	0.33	0.33	0.34	0.35
$[\alpha]_D$ in H_2O (°)	+93.4	+92.0	+94.6	+80.4	+72.0	+38.0	+37.6	+37.4	+37.6	+29.2
Optical density (2.5% w/w)	0.13	0.09	0.11	0.19	0.21	0.09	0.08	0.09	0.06	0.08
Tannin (% w/w)	0.39	0.49	0.51	0.48	0.56	0.77	0.45	0.43	0.44	0.44
Acid equivalent weight	832	1124	976	1659	1274	978	1199	691	663	703

Table 2
Range and average of the properties of *Acacia drepanolobium* and *Acacia kirkii* ssp. *kirkii* var. *kirkii* gums

Parameter	*Acacia drepanolobium* (five samples)			*Acacia kirkii* ssp. *kirkii* var. *kirki* (five samples)		
	Range	Mean	Standard deviation	Range	Mean	Standard deviation
Moisture (% w/w)	12.40–14.64	13.53	1.011	13.11–15.80	14.26	1.136
Acid-insoluble matter (% w/w)	0.25–0.78	0.47	0.2413	0.11–0.82	0.29	0.299
CWIG (% w/w)	1.13–1.62	1.31	0.225	0.66–3.46	1.47	1.169
HWIG (% w/w)	0.61–1.10	0.86	0.195	0.52–1.94	1.08	0.614
Methoxyl (% w/w)	0.84–0.92	0.88	0.031	0.39–0.47	0.44	0.032
Nitrogen (% w/w)	0.48–0.49	0.49	0.00	0.33–0.35	0.34	0.00
$[\alpha]_D$ in H_2O (°)	(+72.0)–(+94.6)	+86.48	9.886	(+29.2)–(+38.0)	+35.96	3.785
Tannin (% w/w)	0.39–0.56	0.49	0.062	0.43–0.77	0.51	0.148
Viscosity, 100 g/l solution	2.32–3.54	2.89	0.443	3.03–5.82	3.81	1.137
Acid equivalent weight	832–1659	1173	317.76	663–1199	847	234.52

Table 3
Metal content in *Acacia drepanolobium* and *Acacia kirkii* ssp. *kirkii* var. *kirkii* gums

Sample	Metal content												
	% w/w				ppm								
	Na	K	Ca	Mg	Fe	Zn	Pb	Cu	Ni	Cd	Mn	Cr	Co
AD I	0.079	0.159	0.607	0.058	81.8	28.5	19.2	6.34	0.000	0.332	8.18	0.000	BDL
AD II	0.086	0.199	0.579	0.061	33.6	24.6	18.3	6.34	0.000	0.633	10.5	0.000	BDL
AD III	0.082	0.133	0.638	0.068	56.5	18.9	13.9	1.01	0.000	0.037	10.7	0.000	BDL
AD IV	0.083	0.168	0.522	0.083	47.0	12.9	16.6	3.63	0.385	0.000	10.0	0.000	BDL
AD V	0.063	0.153	0.654	0.082	75.8	59.8	8.54	4.47	0.000	0.039	14.2	0.012	BDL
KK I	0.117	0.603	0.887	0.058	36.6	17.3	19.5	34.2	3.00	0.449	4.08	1.87	BDL
KK II	0.118	0.512	0.920	0.060	25.5	22.1	7.55	13.4	0.377	0.377	3.58	2.64	BDL
KK III	0.118	0.527	0.885	0.067	24.7	484	13.8	6.43	7.48	0.561	5.47	2.24	BDL
KK IV	0.111	0.335	0.954	0.066	41.0	20.3	12.0	6.18	3.87	0.541	4.06	1.55	BDL
KK V	0.085	0.333	1.229	0.033	21.2	8.72	13.5	4.94	1.19	0.756	0.558	4.38	BDL

AD = *Acacia drepanolobium* and KK = *Acacia kirkii* ssp. *kirkii* var. *kirkii*.

Table 4
Average level of some metals in Tanzanian *Acacia drepanolobium* and *Acacia kirkii* ssp. *kirkii* var. *kirkii* gums

Sample	% w/w				ppm							
	Na	K	Ca	Mg	Fe	Zn	Pb	Cu	Ni	Cd	Mn	Cr
A. drepanolobium	0.079	0.162	0.600	0.070	58.9	28.9	15.3	4.36	0.077	0.208	10.7	0.002
A. kirkii ssp. *kirkii* var. *kirkii*	0.110	0.462	0.975	0.056	29.8	111	13.3	13.0	3.18	0.537	3.55	2.54

should be almost completely soluble in water. The insoluble fraction comprises the acid-insoluble matter and insoluble gel, which partially dissolves on boiling. Cold water-insoluble gel (CWIG) and hot water-insoluble gel (HWIG) are the proportions of the gum that do not dissolve in cold and hot water, respectively, even after prolonged contact with water. The insoluble matter, which includes bark, twigs, sand is not important because it can largely be minimized by hand-grading. Three of the gum exudate samples from *A. drepanolobium* shows (Table 1) similar values of CWIG and HWIG, whereas samples AD IV and AD V had higher values. For the gum exudates from *A. kirkii* ssp. *kirkii* var. *kirkii,* one of the samples, KK V, recorded a CWIG of 3.46% w/w. This is more than three times the average (0.98% w/w) of the other four samples, causing the mean for CWIG for the gum samples from *A. kirkii* ssp. *kirkii* var. *kirkii* to rise to 1.47% w/w. As expected, all the gum samples studied showed a higher value of CWIG than of HWIG. The mean of the CWIG values (1.31% w/w) for the gum exudates from *A. drepanolobium* was somewhat higher than that obtained previously (0.3% w/w) (Mhinzi & Mosha, 1996) for the same species, but much lower than the value (insoluble fraction = 18.6% w/w) obtained previously by Anderson and Karamalla (1966) for *A. drepanolobium* gum from Tanzania. The mean CWIG and HWIG values obtained for *A. drepanolobium* and *A. kirkii* ssp. *kirkii* var. *kirkii* were much lower than those obtained previously for some authenticated gum exudates from Tanzania, for example *Acacia malacocephala* gum (CWIG, 5.88% w/w; HWIG, 3.92% w/w) (Mrosso, 1996) and *Acacia tortilis* ssp. *spirocarpa* gum (CWIG, 13.3% w/w; HWIG, 11.6% w/w) (Mhinzi & Mrosso, 1995).

It has been suggested by Phillips, Pass, Jefferies, and Morley (1980) that the viscosity of some tree exudate gums, particularly gum ghatti, is influenced by the proportion of the insoluble gel fraction. Gum ghatti has been shown to contain an insoluble gel fraction, varying from 8% to 23% w/w, and its viscosities increase with an increase of the insoluble fraction. A similar behaviour has been reported for the gum exudate from *Acacia polyacantha* ssp. *camplyacantha* whose viscosity (35.63 cP for 100 g/l solution) is influenced by its high value of insoluble gel fraction (CWIG = 36.67% w/w) (Mhinzi & Mrosso, 1995). The viscosity of the gum samples from *A. drepanolobium* (Table 1) are quite similar and are consistent with a low value of the insoluble gel fraction. For he *A. kirkii* ssp. *kirkii* var. *kirkii* gum samples, however, one of the samples (KK IV) had a high (5.82 cP for a 100 g/l solution) viscosity. This is more than one and a half times the average (3.30 cP for a 100 g/l solution) of the other four samples. The mean value (2.89 cP for a 100 g/l solution) of the viscosity of *A. drepanolobium* gum found in this work is similar to that reported previously (4.01 cP for a 100 g/l solution) (Mhinzi & Mosha, 1996) for one sample of the same species.

Acacia gums are arabinogalactan proteins and the nitrogen occurs in the form of amino groups (–NH_2) on the terminal amino acid residue of the protein and the peptide linkages (–NHCO–). JECFA/FAO (1990) introduced a specification for nitrogen content (0.26–0.39% w/w) in the definition of gum arabic to ensure identity and purity of the gum. However, this specification was subsequently removed (JECFA/FAO, 1999) after some workers (Mocak et al., 1998) observed that, in fact, intra-species variation of the nitrogen content existed. Tables 1 and 2 show that the nitrogen contents of the gum samples from *A. drepanolobium* and *A. kirkii* ssp. *kirkii* var. *kirkii* do not show significant intra-species variation. However, the nitrogen contents of the two species are different, indicating a difference in the molecular structure of the gum exudates.

Methoxyl content is considered as a useful parameter for distinguishing gums from different species. Gums from members of the series Gummiferae are known to have methoxyl contents in the range 0.47–2.4% w/w, whereas gums from members of the series Vulgares have methoxyl contents ranging from 0.25% to 0.75% w/w. Members of the subseries Juliflorae are known to exude gums with high levels of methoxyl, ranging from 1.2% to 1.68% w/w. The mean methoxyl contents of the gum exudates from *A. drepanolobium* and *A. kirkii* ssp. *kirkii* var. *kirkii* (0.88% and 0.44% w/w, respectively) are within the range expected for gums from the series Gummiferae and no intra-species variation was observed for this parameter.

Biswas, Biswas, and Philips (2000) have suggested that the specific optical rotation of a polysaccharide exudate gum is a linear function of the carbohydrate composition and that exudate gums from a particular species and genus can be represented by a formula, called a rotation operator. The JECFA/FAO (1999) specification requires that gum arabic for food and pharmaceutical applications should be laevorotatory. Gum exudates from species of the series Gummiferae can be readily distinguished from those obtained from species of the series Vulgares by their specific rotation. Members of the Gummiferae series exude dextrorotatory gums normally with optical rotation between +51° and +108° while those from the series Vulgares exude laevorotatory gums with specific rotations between −12° and −30°. Exceptions to this observation include *A. macracantha* with specific rotation (Martinez, de Pinto, & Rivas, 1992) −6° to −18°, although the gum is from the series Gummiferae. As expected, all the gum samples studied in this work have been found to be dextrorotatory. Table 1 shows that the specific rotations of the authenticated gum samples studied exhibit intra-species variation. Thus, *A. drepanolobium* gum samples from Kibigiri, Singida (AD IV and AD V) have lower specific optical rotation values (+80.4° and +72°, respectively) as compared to those from Kintinku, north-west of Dodoma (AD I-AD III) (+92.0° to +94.6°). Likewise the *A. kirkii* ssp. *kirkii* var. *kirkii* gum sample obtained from Chalinze village, 52 km eastward of Dodoma, has a specific rotation of +29.2°, which is different from the other four samples (+37.4° to +38°) obtained 38 km west of Dodoma. Duvallet et al. (1993) reported differences in intrinsic viscosities (between 0.14 and 0.60 dl/g), specific optical rotation (between −25° and −62°) and nitrogen contents (between 0.12% and 0.57 %) in gum exudates from plants of the same species, obtained from the same experimental field, and that the parameters changed with mechanical grinding or moderate heating. This exemplifies the complexity of the molecular structure of *Acacia* gums, as reported by Dror, Cohen, and Yerushalmi-Rozen (2006). A report by Idris et al. (1998) on eight authenticated *A. senegal* gum samples, obtained from two different locations in Sudan, showed no significant variation of the specific rotation values. The specific rotations of *A. drepanolobium* gum samples found in this work (+72° to +94.6°), on the other hand, resemble those from gum exudates of the series Gummiferae, for example, *Acacia sieberana* var. *sieberana* (+103°). However, the data for *A. kirkii* ssp. *kirkii* var. *kirkii* gum samples (+37.4° to +38.0°) (Table 1) are below that reported (+54°) by Jurasek, Kosik, and Phillips (1993) for the same species, but similar to that reported from a single sample (+34.8°) by Mrosso (1996).

Some *Acacia* species are widely grown in Africa for the commercial production of tannin, to be used in tanning industries. These are, by their origin, the Australian species belonging to the series Botryocephalae. *Acacia* species of the series Gummiferae are also known to produce tannin, but in smaller amounts, whereas gum exudates from *Acacia* species of the series Vulgares are believed to be free of tannin. In the Gummiferae species, the gum is easily contaminated with tannin and, as the gum ages on the tree, the tannin content is believed to increase. Previous work by Mhinzi and Mrosso (1995) has shown that gum exudates from some *Acacia* species of the series Vulgares from Tanzania also contain small amounts of tannin. In this study, the tannin content of *A. drepanolobium* gum varied from 0.39% to 0.56% w/w whilst those of *A. kirkii* ssp. *kirkii* var. *kirkii* ranged between 0.43% and 0.77% w/w. Examination of Table 1 reveals that, for the *A. kirkii* ssp. *kirkii* var. *kirkii* gums, sample KK I, which was obtained from the same area as samples KK II and KK III, has a higher (more than one and a half times) tannin content than the average value for KK II-KK V. The values are, however, similar to those reported previously for *A. polyacantha* ssp. *campylacantha*, 0.58% w/w, *A. senegal* var. *leiorhachis*, 0.37% w/w and *A. tortilis* ssp. *spirocarpa*, 0.50% w/w, from Tanzania (Mhinzi & Mrosso, 1995). 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 (Anderson, Bridgeman, Farquhar, & McNab, 1983).

Potentiometric titration furnished the natural pH, percentages salt form of anionic groups and acid equivalent weights of the 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 AEW of *A. drepanolobium* gum samples (range 832–1659). The two samples from Kibigiri village, 84 km east of Singida, recorded higher values of AEW than the three samples from Kintinku village, 230 km west of Singida, suggesting that the variation of the AEW may be ascribed to geographical location.

The maximum limit for total ash for food and pharmaceutical quality *Acacia* gum has been specified by JECFA/FAO (1999) to be 4.0% w/w. Whereas all the gum samples from *A. drepanolobium* met this specification, three samples of *A. kirkii* ssp. *kirkii* var. *kirkii* exceeded the limit. Table 3 shows the metal composition of the studied gum samples. It has been reported by Anderson and Morrison (1989), Chikamai and Banks (1993) that metal ion content in plant material is a function of the composition of the soil on which the plants grow and therefore, may differ in samples obtained from different geographical locations. Calcium ions are known to be responsible for gel formation in some tree exudate gums, such as *Khaya grandifoliola*, but this effect has not been reported for *Acacia* gums. Work by Kunkel, Seo, and Minten (1997) has also shown that gum arabic does not bind added magnesium.

In conclusion, this work has shown that some physicochemical parameters of gum exudates from *A. drepanolobium* and *A. kirkii* ssp. *kirkii* var. *kirkii* from Tanzania show intra-species variation. This is also supported by the high standard deviation of some of the parameters (Table 2). This behaviour has also been observed in gums from *A. senegal* var. *senegal* and *A. seyal* var. *fistula* from Tanzania (Mhinzi, 2003). The inter-species variations are well documented and mean that the molecular structures of gum exudates from different species are different. The intra-species variations presumably result from differences in geographical location. Differences in the properties of gum exudates from a single variant of *Acacia* species have also been reported by Mocak et al. (1998). It has also been reported by Baldwin, Quah, and Menzies (1999) that gums harvested from different subspecies may have very distinct chemical compositions. This work has, therefore, shown that the variation of the properties among the batches of commercial *Acacia* gum from Tanzania may be due to the admixture of gums from different *Acacia* species, as well as intra-species variation of the gum from *A. drepanolobium.*

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