

Multiparameter Analysis of Commercial "Aloe Vera" Materials and Comparison to *Aloe barbadensis* Miller Extracts

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ABSTRACT

Thirty-nine samples of commercial materials represented to be "Aloe Vera" raw materials from 21 sources, were compared to authentic samples of *Aloe barbadensis* gel fillet extracts and whole leaf extracts using a battery of 10 tests. One set of measurements consisted of relatively simple tests, such as alcohol precipitable hexose for detection of total polysaccharides, tests for ionic strength and an HPLC profile used for certification by the International Aloe Science Council (IASC). To verify the results of these tests, the polysaccharide was isolated and analyzed for hexose content and by UV spectroscopy. The crudely isolated polysaccharide was hydrolyzed and sugars analyzed by HPLC using pellicular ion exchange and pulsed amphoteric detection. The isolated low molecular weight fraction was isolated and analyzed for total hexose and reducing sugar. The authentic Aloe used for comparison consisted of 12 consecutively prepared lots of Aloe Research Foundation Standard Samples of *A. barbadensis* gel from 1991 through 1993, and 3 whole leaf extracts from the same period. Only 6 of 21 (29%) commercial "Aloe Vera" powders gave results that were consistent with authentic *A. barbadensis* gel or whole leaf extracts, while the remaining 15 (71%) were found to be either adulterated or completely spurious. Analyses of the 15 samples of powdered "Aloe Vera", showed that 4 samples, derived from 3 separate sources, were adulterated with maltodextrin and the remaining 11 obtained from 9 different sources, were essentially pure maltodextrin. Misrepresentation of powders was easily detected by multiparameter analysis - the materials were usually out of specifications on all three major testing parameters. Six of 13 samples (46%) of "Aloe Vera" liquids appeared to be adulterated. Generally, the spurious feedstock was out of specifications for solids content and the physical nature of the residue in the solids test was abnormal. In one case, the material was totally fraudulent - consisting of maltodextrin and nonionic surfactant. In the remaining five cases, authentic *A. barbadensis* extracts were heavily adulterated with either glycerol and/or nonionic surfactants. Although multiparameter analysis was successful in detecting misrepresented materials, confident diagnosis often required fractionation of the suspect sample. Separate diagnostic trees are probably appropriate for the analysis of powdered commercial materials versus liquid commercial materials purported to be "Aloe". Lastly of all 18 solid or liquid samples consistent with unadulterated *Aloe barbadensis* extract, only 11 (61% of authentic materials, 28% of all materials) retained on HPLC analysis the marker consistent with low levels of bacteria. The IASC Certification Tests (with the addition of a simple assay for total polysaccharide) in conjunction with the Texas A & M University database appear to be quite useful in separating commercial "Aloe Vera" feed stocks consistent with authentic *Aloe barbadensis* from adulterated and spurious materials.

RESUMEN

Treinta y nueve muestras de productos comerciales provenientes de 21 fuentes que pretendían ser materia prima de "Aloe Vera" fueron comparadas con muestras auténticas consistentes en extractos de rebanadas de gel y de hoja entera de *Aloe barbadensis* usando una serie de 10 pruebas. Un grupo de mediciones consistió de pruebas relativamente simples como precipitación de hexosa en alcohol para la detección de polisacáridos totales, pruebas de fuerza iónica y el perfil de HPLC usado para la certificación por el Consejo Internacional de Ciencia del Aloe (IASC). Para verificar los resultados de estas pruebas, los polisacáridos fueron aislados y se analizó su contenido de hexosa mediante espectroscopía de luz ultravioleta. Los polisacáridos aislados en crudo fueron hidrolizados y los azúcares analizados por HPLC usando intercambio iónico de membrana y detección amfotérica de pulso. La muestra auténtica de Aloe usada para la comparación consistió de 12 lotes consecutivos de muestras standard de gel de *A. barbadensis* así como de 3 extractos

de hoja entera preparados de 1991 a 1993 por la Fundación para la Investigación sobre el Aloe. Solo 6 de 21 (29%) de las muestras comerciales de "Aloe Vera" en polvo dieron resultados que fueron consistentes con el gel auténtico o con los extractos de hojas enteras de *A. barbadensis*, mientras que se encontró que las 15 restantes (71%) estaban adulteradas o eran completamente falsas. El análisis de las 15 muestras de "Aloe vera" en polvo, mostró que 4 muestras, derivadas de 3 diferentes fuentes, estaban adulteradas con maltodextrina y que la muestra restante, obtenida de 9 fuentes diferentes, fue esencialmente maltodextrina pura. La falsificación del producto en polvo fue fácilmente detectada por análisis multiparamétricos donde se encontró que los materiales no cumplieron con las especificaciones en los tres parámetros más importantes del examen. Seis de 13 muestras líquidas de "Aloe vera" analizadas (46%) resultaron estar adulteradas. Generalmente, la materia prima adulterada difirió de las especificaciones para contenido de sólidos y presentó una naturaleza física del residuo anormal en la prueba de sólidos. En un caso, el material fue totalmente fraudulento consistiendo de maltodextrina y surfactante no iónico. En los cinco casos restantes, extractos auténticos de *A. barbadensis* estuvieron adulterados considerablemente con glicerol y/o con surfactantes no iónicos. Aunque los análisis multiparamétricos fueron exitosos para la detección de materiales falsos, la diagnosis confiable frecuentemente requirió del fraccionamiento de la muestra sospechosa. Para el análisis de las muestras que pretenden ser "Aloe", probablemente sea apropiado que los esquemas de diagnóstico que se usen para el análisis de materiales en polvo sean diferentes de los que se usen para los materiales líquidos. Finalmente, de todas las 18 muestras sólidas o líquidas consistentes en extracto de *Aloe barbadensis* no adulterado, solo 11 (61% de los materiales auténticos, 28% de todos los materiales) retuvieron el marcador consistente con bajos niveles de bacterias cuando se analizaron por HPLC. Las pruebas de certificación del IASC (con la adición de un simple ensayo de polisacáridos totales) en conjunción con la base de datos de la Universidad de Texas A&M resultaron ser bastante útiles para la separación de materiales adulterados o falsos de materiales crudos comerciales de "Aloe Vera" que si consisten en auténtico *Aloe barbadensis*.

Key Words: Polysaccharide; maltodextrin; alcohol precipitable solids; E Peak

The "Aloe Vera" industry, which markets products derived from *Aloe barbadensis* Miller, consists mostly of small and medium sized privately held enterprises and only a handful of large integrated companies. Most companies occupy a single niche - they are either producers of raw Aloe juices, juice processors, brokers of processed juices, or manufacturers of consumer products whose lines are heavily dedicated to drinks or cosmetics containing 100% "Aloe". Few of these companies publish figures on yearly production or sales, making it impossible to determine the yearly magnitude of sales of "Aloe" with precision. The International Aloe Science Council (IASC) is an industry group comprising almost 150 companies dealing with "Aloe" in its various aspects. The IASC estimates that sales of "Aloe" raw materials feed stocks are in excess of \$20,000,000 per year. Over \$100,000,000 is spent yearly by U.S. consumers on "100% Aloe" drinks and cosmetics. Market analyses estimate that consumers spend in excess of one billion dollars a year on products that "contain Aloe". All of these purchases occur in a quasi "health food" atmosphere, most "Aloe" companies are strongly opposed to any form of regulation. "Tests" and "testimonials" from pseudoscientists are frequent. Under these circumstances there is a high potential for consumer fraud at all levels of production and distribution. In addition, the lack of available information on the quality and composition of the Aloe used in the products has been a major obstacle to scientific studies that attempt to document biological activities attributed to this plant and to isolate the active agents in Aloe. Failure to reproduce experimental results, even within the same laboratory because of differences in materials reported to be "Aloe" have severely damaged the reputation of the Aloe Industry in the mainstream scientific community.

As part of our studies on biological activities of *Aloe barbadensis* gel extracts (Strickland et al. 1994, Byeon et al. 1998) it became necessary to better define the chemical composition of Aloe initially described by Waller et al. (1978). Concurrently, Wang and Strong (1995) were studying the extracts of fresh leaves. Therefore, we coordinated our efforts with those of Wang - this group focusing on the chemical composition of commercial "Aloe" materials. We initially utilized the IASC Certification tests (Science and Technical Committee, 1992). We developed ancillary tests especially suited for industrial materials and the nature of the adulterants we found in commercial "Aloe". We explore herein the suitability of the IASC Certification analyses for testing commercial materials for fraud and examine which further tests are useful for the confirmation of the diagnosis of adulteration. By better defining the composition of commercially available Aloe, insight is gained into the nature of one source of experimental variability in biological assays.

MATERIALS AND METHODS

Aloe Research Foundation Standard Samples of Aloe barbadensis. A key to this study was the use of Aloe Research Foundation (ARF) materials which provided reasonably large quantities of lyophilized Aloe of defined provenance against which to compare the commercial materials. These materials came from one of the four plantations which were studied in the Texas A & M University study (Wang and Strong, 1995). In all cases industrial equipment and personnel was used to produce the Standard Samples under controlled conditions. Because of the small scale (generally ~215 liters) or the preparations, meticulous attention could be given to

sanitation and rapidity of processing. The result of this is that, in crude gel, levels of bacteria (generally <10,000 organisms per ml) and anthraquinones (generally <50 ppm) were lower and levels of polysaccharide were higher than in comparable industrial materials. The ARF reference statistical sample used in this study consisted of "Process A" gel (for exemplification of processing see - Pelley et al., 1993; Waller et al., 1994). These materials were subjected to no processing other than passage through a stainless steel pulp remover (250 μ m) followed by lyophilization. The time interval between leaf harvest and lyophilization was generally 4 hours. Table 1 gives the characteristics for these powdered reference samples for the analytes: electrical conductivity, percent mass of alcohol precipitate, percent alcohol precipitable hexose, and content of malic acid, known in the Industry as "E Peak".

For calibration of the HPLC test for "E Peak", different ARF materials ("Process E") were used. In this case (Pelley et al, 1993; Waller et al, 1994) depulped gel was pasteurized, decolorized with activated charcoal, filtered using diatomaceous earth and cellulose filters, concentrated by rising/falling thin film evaporation and lyophilized. This material essentially corresponds to industrial "Freeze Dried" material of the highest quality. A new standard is prepared every six months and this material is generally employed as the reference standard for IASC Certification.

Sample Acquisition, Study A. Thirty-two samples of commercial "Aloe Vera" materials suitable for analysis were received by Stephens and Associates, an independent testing laboratory. These were received either from the vendors of the feed stock "Aloe" materials or from manufacturers of consumer products. Those "Aloe" materials received from consumer product manufacturers were almost invariably coded as to conceal the identity of the feedstock vendor and were transmitted in containers other than those originally supplied by the feed stock vendor. In such cases, only the samples unequivocally labeled as to claim of contents were included in the 32 samples studied herein. As a condition of the study consumer product manufacturers were promised anonymity. In study A, due to its nature, an intact chain of evidence was not maintainable nor were all testers blinded. After testing of samples for conductivity and total polysaccharide at Stephens and Associates, the remaining material was transferred to UTMB for further analysis. A portion of this remaindered sample was coded and sent to TAW for HPLC analysis for "E Peak". While the samples sent to the UTMB facility were coded by the Consumer Products Manufacturers, the investigators were in possession of the results of analyses conducted by Stephens and Associates. Thus, the UTMB analyses were performed as a blinded but not a double-blind Study.

Samples Acquisition, Study B. Based on Study A, vendors of raw materials were selected for Study B. Many of the details of the design, implementation and results of Study B were reported by Dr. Nicholas Schmidt at the 1995 Annual Meeting of the International Aloe Science Council. Ten samples were received by Inchcape Testing Service, an independent testing laboratory. They received these materials through an independently contracted third party otherwise unassociated

with this study. In all cases the chain of evidence was maintained intact and all samples were coded by Inchcape Testing Service such that testing was done in a blinded fashion. Seven samples consisted of commercial "Aloe Vera" materials. The unopened product samples were submitted by the independently contracted third party to Inchcape Testing Service, logged in and assigned a code number. None of the personnel who prepared and coded the samples communicated with those persons testing the samples. Three samples consisted of reference standards: 2 new ARF Standard Samples of *Aloe barbadensis* (one of gel and one of whole leaf) and one of commercial maltodextrin (Amaizo, Inc., Low Dex 10, lot E3186-135). These were transmitted directly to the independent testing laboratory by the manufacturers and were coded in a manner similar to the test samples. Inchcape Testing Service then performed the IASC Certification Tests on all ten powders. Inchcape Testing Service also performed the alcohol precipitable hexose test for polysaccharide on all ten samples. The alcohol precipitate was then resolubilized in water. These solutions, coded in a manner such as to conceal their nature, origin and original physical properties, were then transferred to the UTMB laboratory for further analysis of polysaccharide. Thus, these results were produced in a double-blinded study.

Determination of IASC Parameters. For the ten samples of Study B, Inchcape Testing Service performed the tests using the same protocols they employ for IASC Certification (Science and Technology Committee, 1992). Determination of metal cations was by inductively coupled plasma emission spectrophotometry. HPLC was performed using IASC conditions as we have previously described (Pelley et al., 1993 or Waller et al., 1994). Since the identity of "E Peak" as malic acid was proprietary to the IASC at the time, the method was calibrated using the current AHF Standard Sample for Gel. Data for "E Peak" levels in Study A are expressed as Normal (80 to 190% of the ARF Process E Standard Sample), Low (5 to 10%), or Absent (less than 5%). Data for Study B are expressed as percentage of the ARF Standard.

Determination of Conductivity and Alcohol Precipitable Hexose (Crude Total Polysaccharide). Powdered "Aloe Vera" or ARF Standard Samples (62 mg) were dissolved in water (10 ml) to a concentration approximating the average concentration of solids in fresh Aloe gel (Wang and Strong, 1995). Electrical conductivity was determined between clean platinum electrodes spaced 1 cm apart using any one of a variety of instruments. All data are expressed in microSiemens. Interlab correlation is excellent ($R^2 > 99\%$) as is to be expected from a primary physical-chemical measurement.

The sugars of *Aloe barbadensis* consist almost exclusively of hexoses (Waller et al., 1978). These are in two forms: monosaccharides (95% glucose, 5% fructose, R.P. Pelley et al. unpublished observations) which comprise approximately 20% of the solids content and polysaccharides (85% mannose 1 7% glucose, and 4% galactose) which comprise approximately 10% of the mass of solids. Polysaccharide is traditionally defined as that proportion of saccharide which is sufficiently large to be precipitable with alcohol. Therefore, alcohol precipitation is employed to separate large, hexose-containing

polysaccharide from small, hexose-containing monosaccharides (fresh *Aloe barbadensis* gel contains very little disaccharide or oligosaccharide). With Process A ARF materials, the separation of polysaccharide and monosaccharide by alcohol is quite clean. Therefore, we consider alcohol precipitable hexose to be essentially the same as polysaccharide.

In order to determine total crude polysaccharide, "Aloe" powders or ARF Standard Samples (500 mg) were dissolved in 5 to 20 ml of water (depending on solubility). Then, crude polysaccharide and a complex of divalent cation/multivalent carboxylic acids was precipitated by addition of ethanol to 80% (v/v) concentration and incubation overnight at 4°C. Precipitate was separated from supernatant by centrifugation at 3,000 x G for 15 minutes followed by decantation. The precipitate was dissolved in 5 to 20 ml of water (depending on solubility). The hexose content of the precipitate was determined by the Phenol Sulfuric Acid method (Dubois et al., 1956) using mannose as a standard. Data on crude total polysaccharide levels are expressed as the percentage of total solids that are alcohol precipitable hexose. In the case of liquid samples, these materials were first lyophilized and then 500 mg of solid material was tested.

Isolation of Crude Polysaccharide Fraction. For Study A crude polysaccharide was isolated by exhaustive dialysis of crude materials through SpectraPor #3 tubing (MW cutoff ~1,800 daltons). For the 32 samples in Study A, 10 g of

commercial powder (or 10 g of lyophilized material from liquid samples) was available. This was dissolved in water to a final volume of approximately 25 ml and dialysed against 250 ml of water purified by reverse osmosis ($\mu < 1 \mu\text{Siemens}$). Dialysis was conducted at 4°C with the dialysis buffer changed every 24 hours until the conductivity of the dialysate had been $< 100 \mu\text{Siemens}$. This fraction corresponded very closely ($> 90\%$) to polysaccharide in the case of authentic *Aloe barbadensis* gel as evidenced by the total hexose content and sugar composition upon hydrolysis. In the case of maltodextrins, this method produced significant amounts of alcohol-soluble oligosaccharides. As expected for limit hydrolysates of corn starch, the oligosaccharide to polysaccharide ratio varied from maltodextrin to maltodextrin.

In Study B a greater degree of polysaccharide purity was desired. Therefore samples were first dissolved in water and precipitated with alcohol. Then the alcohol-insoluble fraction was dissolved and dialysed. The alcohol precipitations were done at Inchcape Testing Service and the resolubilized precipitates transferred to UTMB where they were lyophilized. After reservation of approximately 10 mg (generally about 5-10%) of the lyophilized sample, the bulk of the sample (160 to 300 mg) was dissolved in 5 ml of water and dialysed. Alcohol precipitates were dialysed twice using Spectra Por tubing with a 5,000 dalton cutoff against a 250 ml water (a 50 fold excess) for two 24 hour cycles to a final ionic strength of $\leq 100 \mu\text{Siemens}$ (dialysate). The correlation coefficient between the amount of polysaccharide measured by the alcohol precipitable hexose

Table 1. Basic analytical parameters of Aloe Research Foundation gel fillet standard samples.

Standard Sample ^a	Conductivity $\mu\text{siemens}^b$	EtOH Precipitable Solids ^c	Total Polysaccharide ^d	HPLC Analysis ^d
	μS	%	%	
91A	2,356	30.6	7.4	Normal
91D	1,488	23.0	6.5	Normal
92A ₂	2,108	30.2	8.7	Normal
92B	2,232	21.6	5.6	Normal
92C ₁	1,054	45.8	13.5	Normal
92C ₂	1,354	38.4	13.2	Normal
93A	2,170	40.2	11.3	Normal
93B	2,900	47.2	9.7	Normal
93D ₁	2,050	38.0	6.9	Normal
93D ₂	2,350	33.6	9.1	Normal
93E ₁	2,200	37.0	9.8	Normal
93E ₂	2,100	35.6	8.9	Normal
Mean	2,030	35.1	9.2	
Std. Dev.	502	7.9	2.5	
S.E.M.	145	2.3	0.7	
Coef. Var.	24.7%	22.5	27.1	

^aARF'91D, 91D, 92A₂, 92C₁ and 92C₂ were prepared from leaves at the Lyford Plantation. ARF'92B, and all of the samples from 1993 were prepared from leaves from the Gonzalez Plantation. These materials do not appear to differ significantly from each other. ARF'92C₁ consisted of leaves from young plants while '92C₂ was prepared from older plants. ARF'93C₁ and ARF'93E₁ were filleted by hand while ARF'93C₂ and ARF'93E₂ were filleted by machine. Aside from a slight difference in alcohol precipitable solids, there does not appear to be a difference between the materials.

^bSolution at 0.62 g/dl (actual 62 mg in 10 ml).

^c500 mg of lyophilized powder was dissolved and precipitated with 80% EtOH. The solids content (Alcohol Precipitable Solids) of the resolubilized pellet was determined by lyophilization. The total hexose content was determined by Phenol Sulfuric Acid assay which yields the values for Total Polysaccharide hexose

^dDetermined by the IASC method.

assay and the mass of polysaccharide isolated by this method was 0.967 (R^2).

Analysis of Isolated Polysaccharides. First the content of total hexose (Phenol Sulfuric Acid method, Dubois et al, 1956) and reducing sugar (Neocuproine method, Dygert, 1965) was determined. Then the absorbance of the polysaccharide ultraviolet wavelengths was determined by scanning with a Hewlett Packard Model 8452A spectrophotometer.

Next the polysaccharide was hydrolysed. Between 1.5 to 3 mg of dry, crude polysaccharide was placed into a hydrolysis tube (Pierce Inc., Rockford, IL) and 1.0 ml of hydrolysis grade constant boiling HCl (Pierce) was added. After dissolution, a 0.5 ml aliquot of sample was withdrawn as a pre-hydrolysis control. The hydrolysis tube was degassed by high vacuum while chilled in an ice bath (to minimize loss of HCl). The tube was sealed and hydrolysis accomplished by incubation for 30 minutes at 120°C. In all cases, before further assay, the HCl was neutralized with concentrated NaOH. The degree to which sugar is destroyed during hydrolysis was monitored by total hexose assay (Phenol Sulfuric Acid method, Dubois et al. 1956) of pre-hydrolysis control and post hydrolysis material. As a laboratory control, authentic *Aloe barbadensis* gel polysaccharide, purified in the UTMB laboratory, was analyzed in parallel.

Following hydrolysis, neutralization and total hexose assays, the samples were adjusted to a concentration of 20 µg hexose/ml by addition of water and filtered through submicron filters before injection. The composition of monosaccharides was determined upon 25 µl samples using the Dionex BioLC HPLC sugar analysis system. This consists of a Dionex Ion Pac pellicular NH₄⁺ ion exchange column using a minimal carbonate NaOH buffer system. Quantitation of sugars was by Dionex pulsed amphoteric detection with 4290 Integrator ensuring high sensitivity and specificity for sugars. Calibration on the days when samples were run consisted of runs of authentic sugar standards. For each series of analyses (usually 8 to 10 experimental samples), between 4 to 6 calibrations were carried out. Agreement between retention times of standard sugars and assignments for experimental sugars were: glucose, 98%; mannose, 97%; and fructose, 98%. Galactose was usually assigned based on its position relative to glucose (which was present in all samples). Data on the sugar composition of hydrolysed crude polysaccharides is expressed as a percentage of individual sugars versus total sugars detected by the pulsed amphoteric detector.

Analyses of Dialysates. In Study A, the dialysates from isolation of crude polysaccharides were lyophilized and total hexose content and reducing sugar content determined by the same assays as described above. In certain selected samples, the monosaccharide composition of the dialysates were analyzed by HPLC using the Dionex system. The calibration for these analyses were carried out in the same manner as above.

RESULTS

Composition of ARF Gel Standards. In order to determine how closely commercial preparations of "Aloe Vera" resemble authentic *A. barbadensis*, standards were prepared from

multiple lots of gels fillets and analyzed using a variety of tests. Aloe Research Foundation (ARF) Standard gel samples were used because these materials were prepared under rigorously controlled conditions. The properties of 12 separate lots of ARF Standard samples were examined using tests for conductivity, solids, polysaccharide and HPLC analysts for "E Peak" (malic acid). The results are shown in Table 1. Despite the fact that the different lots of gel were prepared over a 2 year period and the leaves used were obtained from 2 different plantations the variability between the different lots of gel was remarkably low. All samples tested had normal levels of "E" Peak based on International Aloe Science Council (IASC) criteria. While the conductivity of the gel ranged from 1,054 µS to 2,900 µS, ethanol precipitable solids and total polysaccharide content varied by only 2 fold. Differences between the lots of gel could not be attributed to factors such as the age of the plants, source of the leaves or method of filleting.

Analysis of Commercial "Aloe Vera" Powders - Study A. Samples of 19 commercial powders purported to be "Aloe Vera" were analyzed and the results compared with authentic Aloe from the ARF Process A standards or commercial maltodextrin. The results are presented in Table 2. The columns of the table are divided into three sections: (i) results of tests based on either the IASC Certification parameters (Conductivity and HPLC) for small molecules, and a test for the commonest large molecule, Polysaccharide Content, performed using unfractionated material; (ii) isolation and analysis of crude polysaccharide (Material greater than 10,000 MW); and (iii) analysis of the low molecular weight material in the dialysates (Material less than 10,000 MW). Sorting the data by these methods gave unequivocal separation into the following three groups: commercial materials 1-7, whose values were consistent with Authentic "Aloe Vera"; commercial materials 8-16, with values were similar to maltodextrin; and commercial materials 17-19 that had values intermediate between authentic "Aloe Vera" and maltodextrin. This last group may represent commercial preparations of authentic "Aloe Vera" to which maltodextrin has been added. In all 19 cases, the label claim of the sample received was consistent with "pure Aloe Vera".

Analysis of Unfractionated materials. Commercial "Aloe Vera" powdered materials 1-7 were obtained from 4 separate sources representing feedstock and consumer products manufacturers. All of the materials in this group had conductivity levels within a 2 standard deviation (σ) limit for the ARF Standard Sample distribution. Thus, the total ionic strength of these materials, adjusted to the standard IASC concentration, could not be distinguished from authentic *Aloe barbadensis* gel fillet extracts. Only one of the commercial "Aloe Vera" samples in this group had a polysaccharide content of 15.9% which was more than 2 σ greater than the Process A ARF Standard Sample. One sample in this group lacked "E Peak". The remaining 6 materials in this group had low (2 of 7) to normal (4 of 7) levels of "E Peak". Based solely upon the IASC diagnostic HPLC profile for "E Peak", the identity of three materials which had absent or low "E Peak" values would have been regarded as questionable. However, in

Table 2. Characteristics of Commercial Powdered Materials Consistent with "Aloe Vera"

Material ^a	Unfractionated Material			Material Greater Than 10,000 MW				Material Less Than 10,000 MW		
	Ionic Strength ^b	Polysaccharide content ^c	HPLC ^d "E Peak"	%Total Material ^e	% Hexose ^f	UV Scan ^g	Glu:Man ^h Gal percentage ^h	Hexose ^f	Reducing Sugar ⁱ	Ratio
Maltodextrin	62	47.1%	Absent	58.6%	89.3	0.01	93:7:0	76.5%	15.3%	0.20
ARF Process A Standards	2,030±145	9.2±0.7%	Normal	17.3±1.7	73±6	1.8±0.4	7:85:4	13.8±1.5	15.5±3.1	0.98
Commercial Samples							1 1 1			
1-7	2405±309	6.1±2%	Low-Normal	4.2±0.9	67.5±2.8	0.6±0.2	7:81:7	14.8±2.4	9.4±1	0.70±0.25
8-16	255±71	39.9±5.6%	Absent	53.2±3.1	85.4±1.9	0.02±0.01	83:8:1	65.1±4.5	11.6±0.3	0.18±0.01
17-19	1,022±22	24.5±5.1%	Absent	35.1±0.5	84.6±3.6	0.06±0.02	70:19:1	43.5±3.0	13.3±1.2	0.31±0.02
							8 12 1			

^aSupplied by three different sources: Feedstock Manufacturers (5 samples), Consumer Product Manufacturer's Representative (1 sample). None of the samples supplied were the same material

^bConductivity of a 0.58 to 0.62 g/dl solution at ambient laboratory temperature. Results for maltodextrin standard are the means for determinations done on four separate occasions. Results for ARF Process A Standard Samples are the means ± S.E.M. for analyses done on twelve different Standard Samples.

^cValues expressed are the % of solids that are alcohol precipitable hexose. Results for ARF Process A Standard Samples are the means ± S.E.M. for analyses done on twelve different Standard Samples.

^dConducted according to the IASC protocol. "E Peak" is graded as Normal in quantity, Low, Very Low but still detectable or Absent.

^eTen grams of powder were dialysed exhaustively using Spectraphor #1 tubing and deionized water. Samples were dialysed for a minimum of three times against at least 5 volumes of water. In all cases the conductivity of the final dialysate was under 100 µSiemens. The data are expressed as percentage retained material (mass/mass.) Results for ARF Standard Samples are the means ± S.E.M. for analyses done on fifteen fractionations.

^fDetermined by the Phenol Sulfuric Acid Assay for total hexose (Dubois Assay). Results for hexose content of ARF Standard Samples, high MW fraction are the means ± S.E.M. for analyses done on ten different fractionations. In the case of the low MW fraction, 13 isolations.

^gSolutions of isolated polysaccharide, at a concentration of 1 mg/ml, were scanned at wavelengths from 200nm to 800nm. Data are expressed as OD_{280nm} (Mean ± S.E.M. of 8 determinations from 8 fractionations).

^hRetained material was hydrolysed in 6N constant boiling HCl for 10 to 20 minutes at 120°C. After neutralization, ratio of monosaccharides determined by HPLC on a Dionex PA-1 column with a pulsed Amphoteric Detector. ARF Standard Sample means are for 15 determinations on separate isolates. Means for commercial materials are for the 4 samples consistent with gel.

ⁱDetermined by the Neocuprione assay for reducing sugar (Nelson Assay). Note: the reducing sugar content of the retained fraction (high molecular weight material) was 5.3±0.3% (Mean±S.E.M.). This value was based on 5 fractionations of 4 ARF Process A Standard Samples of Gel. For these Standard Samples the respective value for total hexose content of the low molecular weight fraction was 15.8±1.5%.

^jWhole leaf preparation, polysaccharide sugar composition is within normal limits when compared to a limited number of ARF whole Leaf Standard Samples. Statistical values for the three samples alleged to be whole leaf are: Glucose, 28±17%; Mannose, 48±16%; Galactose, 14±4%; coefficient of Variance, 67%.

all of these cases, the other two analytical parameters (Ionic Strength and Polysaccharide Content) were within the normal limits for authentic *Aloe barbadensis*. Thus, reliance upon a single test method can be misleading since these materials were not different from authentic Aloe and show no signs of adulteration in two of the three analyses.

Commercial samples 8-16 formed a second group which had ionic strength values that ranged from 64µS to 690µS, well below even the lowest values for authentic Aloe. In all cases the "E Peak" HPLC marker was absent in materials in this category. Furthermore, the polysaccharide (alcohol precipitable hexose) content of all the samples in this category were at least 5 σ above the mean of the ARF Standard Sample. Thus, group II samples resembled maltodextrin rather than authentic *Aloe barbadensis*.

Three commercial samples, 17-19, were placed into a third group because they exhibited values intermediate between authentic Aloe and maltodextrin. For all three samples in this group the ionic strength was almost exactly one half of that of the ARF Standard Samples or commercial samples 1-7. None of these materials contained the diagnostic "E Peak" marker on HPLC. Furthermore, the polysaccharide (alcohol precipitable hexose) of all three materials was on the average, 4 times higher than commercial "Aloe Vera" and almost three times higher than the ARF authentic Aloe standard. Moreover, the polysaccharide content was almost exactly one half that of commercial maltodextrin.

There may be several explanations to account for the intermediate results obtained for commercial samples 17-19. First, the state of nomenclature for commercial "Aloe"

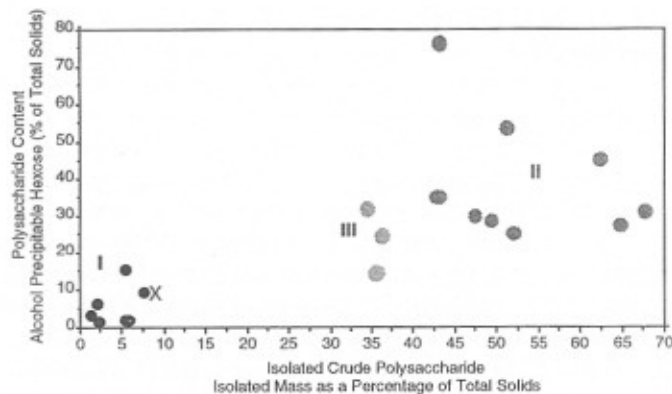


Fig. 1. Correlation between polysaccharide content, determined by alcohol precipitable hexose, and mass of crude polysaccharide isolated by dialysis for the 19 samples in Study A. Three populations, labeled I, II, and III correspond to groups in Table 2. The mean values for 12 ARF Standard Samples is indicated by X. Correlations were statistically significant ($p < 0.0001$) although the "goodness of fit" was only moderate ($R^2 = 0.515$). For simplicity, the line of best fit is omitted

materials is somewhat confused because the IASC uses the designation 200X or 200:1 to designate pure, unadulterated freeze-dried materials containing a negligible mass of preservatives. The terms 100:1 or 100X are used by the IASC to indicate materials manufactured by a spray dried process and therefore containing by the nature of their processing, 50% (by weight of solids to weight of solids) maltodextrin. Therefore, the possibility exists that spray dried 100X or 100:1 might be confused with 100% Aloe. However, inspection of sample labels indicated that no labeling of this type (100X or 100:1) was present. Another explanation may be that these materials consist of freeze-dried "Aloe Vera" adulterated 50% with maltodextrin. In any case, the label representing these agents as pure "Aloe Vera" was not correct.

Analyses of Isolated Crude Polysaccharides. The results above differentiate commercial "Aloe Vera" into three groups; (I) consistent with authentic *Aloe barbadensis*, (II) consistent with commercial maltodextrin with little or no Aloe present, (III) consistent with Aloe adulterated with maltodextrin. In order to confirm the suppositions conjectured by this classification scheme, the crude polysaccharide fraction was isolated from these materials. Comparison of polysaccharide content versus mass of isolated crude polysaccharide (Figure 1) confirmed the existence of 3 distinct groups of materials for the 19 materials in Table 2. One group of commercial materials (indicated in Figure 1 by black dots, designated I) consisted of the materials whose properties were similar to authentic *Aloe barbadensis* (mean of ARF Samples, indicated by X). Category II materials (indicated by the darkly shaded dots in Figure 1, designated II) had a high polysaccharide content by alcohol precipitable hexose assay and large amounts of material were isolated by dialysis. Finally, a third group of

materials (indicated by the lightly shaded dots designated III) wherein approximately 35% of the material was of high molecular weight upon dialysis. This third group of materials had significantly higher total polysaccharide contents (by alcohol precipitable hexose assay) than is usually observed with Aloe and was identical with the materials in Table 2 suspected of being Aloe adulterated with maltodextrin.

The purity of the isolated materials can be best judged by the hexose content (Table 2, column 7). The average hexose content of the polysaccharide isolated by dialysis from commercial materials consistent with "Aloe Vera" compared favorably with the hexose content of polysaccharide isolated from native *Aloe barbadensis* gel in our laboratory by similar methods. It was also not different from the hexose content of polysaccharide isolated in our laboratory by the classical method (Gowda et al., 1979) or from values in the published literature (Gowda et al., 1979).

The dominant polysaccharide in *Aloe barbadensis* is heavily acetylated and extremely hygroscopic (Gowda et al., 1979; Mandal and Das, 1980). Therefore, it is highly unusual to obtain a total hexose content of greater than 75%. Eight of 9 of the polysaccharides isolated from the group II materials above, suspected to be pure maltodextrin, had a hexose content in excess of 80%. Thus, with respect to total hexose content they more closely approximated isolated commercial maltodextrin polysaccharide than Aloe polysaccharide, providing further evidence that the polysaccharides in the second group in Table 2 were indeed related to maltodextrin. Polysaccharides in the third group were similar, suggesting that maltodextrin was present in these samples.

The isolated crude polysaccharides were hydrolysed and examined for sugar composition. Analysis of numerous samples of polysaccharide isolated in our laboratory from the ARF Samples yielded a sugar composition that had a 12:1 ratio of mannose to glucose. This was in good agreement with data in the published scientific (Gowda et al., 1979) and patent (McAnalley, 1990) literature. The average result of the analyses of the "Aloe Vera"-like commercial materials yielded an essentially identical 10:1 ratio. It should be noted that although the means for ARF Standard Samples and commercial materials most consistent with "Aloe" were not different, there was considerable heterogeneity within both groups. The ARF Samples contained individual specimens with mannose to glucose values as low as 6:1 and as high as 25:1. Three samples in group I of commercial samples had lower than expected polysaccharide (3.7% versus 7.2%). After hydrolysis they had lower than expected amounts of mannose (31.8% versus 84.0%) but much higher (13.1% versus 4.1%) amounts of galactose or a galactose related charged sugar. In this respect they more closely resembled the ARF Standard Sample of Whole Leaf Extract than ARF Standard Sample Gel.

Authentic commercial maltodextrin, on the other hand, has a low molecular weight fraction rich (76% versus 14%) in the hexose products of limit digestion of starch with amylases.

As expected from the action of amylase, very little monosaccharide is present (Total Hexose: Reducing Sugar; maltodextrin, 1:0.20). The polysaccharide sugar composition of the second group of commercial materials were uniformly similar - with one exception they are identical to maltodextrin. The commercial maltodextrin commonly used in the Aloe industry is predominately 93% glucose with some (7%) mannose and no galactose. Some samples of maltodextrin appeared to come from high fructose corn (maize) and therefore contained polysaccharide fructose in amounts as high as 28% (data not shown). With the exception of one sample, all of the group II commercial materials had high glucose, low mannose polysaccharides from which galactose (and galactose-related acid sugars) was essentially absent. Finally, examination of polysaccharide composition of the three materials (samples 17-19) in group III suggested that very little Aloe polysaccharide was present. Mannose was present in variable amounts (from trace amounts to 43%, not shown). Furthermore, in all samples in this group, the level of galactose was 1% or less, compared with 4% in the ARF Gel Standard Samples.

In summary, sugar composition of polysaccharides from powders suspect of fraud invariably confirmed a maltodextrin origin for the material and confirms the absence of material consistent with Aloe.

Spectroscopy of Crudely Isolated Polysaccharides.

Infrared Spectroscopy is used as a method of measuring non-polysaccharide materials in Aloe, based upon the patent literature (McAnelley; 1989, 1990A & 1990B). Critical to the interpretation of these spectra (McAnelley; 1989) is discrimination between the carboxyl signal at 1,550 to 1,600 cm^{-1} and the ester absorbance at 1,725-1,750 cm^{-1} . Authentic commercial "Aloe Vera" contains large amounts of the multivalent organic acids oxylate, malate and citrate (commonly added as a preservative) which potentially contribute absorbance at 1,550 to 1,600 cm^{-1} . Unfortunately, their (McAnelley; 1990A & 1990B) usual method of preparation and analysis (identical figures 8 through 13 in both patents) yields in 5 of 6 spectra, the presumed acetate signal at 1,725-1,750 cm^{-1} only as a shoulder of the 1,550 to 1,600 cm^{-1} absorbance peak. This same effect is prominent when the analysis is applied to raw Aloe (McAnelley, 1990A & Fig 14 in 1990B). The results of our FTIR analyses of group I commercial Aloe are essentially identical to theirs. Although FTIR is useful in confirming the presence of polysaccharide esterified with carboxyl-containing compounds, the ability of this method to quantitate "Acemannan" polysaccharide is limited and extremely cumbersome and costly. Therefore, an additional "cross-check" for polysaccharide composition was sought.

The trace amounts of metal cations and organic acids present in even the crudest polysaccharide preparations in this study minimally contribute to the UV spectroscopy at wavelengths $>240\text{nm}$. Polysaccharides, per se, usually also have minimal UV absorbance above 220nm. In Aloe, the large bulk of the UV absorbing materials are the anthraquinones, chromones, terpenes and phytosterols removed by the dialysis process. However, the ARF Standard Sample polysaccharides possessed a significant absorbance shoulder at approximately 280nm (Figure 2). This is not due to the presence of the amino

acid tyrosine as the amino acid content of Aloe polysaccharides is vanishing low ($<0.1\%$ by hydrolysis and amino acid analysis by Beckman analyzer, data not shown). Similarly, this 280 nm absorbance is not due to small molecules with aromatic groups since these are ethanol and water soluble, as observed in Figure 2. Rather, it appears that the UV absorbing residue is covalently linked to the polysaccharide (data not shown).

On a statistical group basis, absorbance at 280 nm offers significant advantage in discriminating *Aloe barbadensis* products from maltodextrin. Analysis of eight ARF Standard Sample preparations of crude polysaccharide had significant absorbance (Table 2, Column 8). Polysaccharides isolated from commercial materials consistent with authentic Aloe by multiple parameters, also had significant UV absorbance (minimum 0.2 OD₂₈₀, 1 mg/ml solution). Polysaccharide isolated from commercial maltodextrin lacked such absorbance. Similarly, commercial materials whose polysaccharides were consistent with maltodextrin also failed to absorb UV light. We have already demonstrated that UV spectral analysis is crucial to differentiating the adulterants commonly observed in liquid Aloe materials. Thus, this method offers a low cost alternative to characterizing the polysaccharide composition of purported Aloe materials.

Analysis of Small Molecules - Total Hexose and Reducing Sugars

Analysis of the sugars in the low molecular weight fraction (dialysate) of ARF Standards and maltodextrin revealed that only approximately 14% of the solids content of authentic Aloe was hexose (Table 2, column 9). Mineral cations, chloride, and organic acids make up most of the remaining mass. In contrast, the majority (76.5%) of the small molecules in maltodextrin were hexose. Commercial materials, which are consistent with authentic Aloe by other analyses, had a hexose content nearly identical to the ARF Standards. In contrast, commercial samples 8-16 which exhibited maltodextrin-like properties by other criteria and also resembled maltodextrin in their low molecular weight hexose content. Samples 17-19 that were consistent with adulteration with maltodextrin were intermediate between maltodextrin and authentic Aloe by this test.

Analysis of reducing sugar revealed that essentially all the carbohydrate present in Aloe is reducing sugar (ARF, Process A Standard Samples contain very minimal amounts of oligosaccharide). Dionex HPLC sugar analysis revealed the sugar composition was 95% monosaccharide glucose with fructose comprising the remainder (data not shown). These results are consistent with most plant juices with the exception of sugar cane or sugar beets, whose juice is rich in the disaccharide sucrose.

Analysis of Commercial "Aloe Vera" Powders - Study B.

Unfractionated materials. It was possible that the crucial assertions of Study A - that some commercial "Aloe Vera" was being heavily adulterated, misrepresented or that materials were totally fraudulent - could be the results of (i) errors by the manufacturers, (ii) mixups or tampering during the transmission of samples from those collecting them to the testing lab or (iii) errors or tampering during the Study A testing process (which was not blinded). Therefore, to resolve these issues, a second study was initiated using samples of

Table 3. Characteristics of commercial powdered materials of defined provance.

Sample No. ^a	Unfractionated Material				Ionic Strength	HPLC ^d "E Peak"	Polysaccharide Content ^e	Alcohol Ppt's Solids ^f	Isolated Crude Polysaccharide	
	Metal Cation Content ^b								% Total Material ^g	Glu:Man:Gal percentage ^h
	Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺						
1 ARF-Gel	238	95	410	31	2,960 μ S	103%	103%	34.2%	5.4%	8.4:81.1:1.6
2	188	103	411	30	2,960 μ S	124%	6.36%	33.2%	4.8%	3.4:93.5:2.5
3	39	206	274	102	1,870 μ S	142%	9.3%	36.8%	7.1%	7.1:89.8:2.1
4 ARF-WLE	128	285	445	51	2,570 μ S	186%	0.99%	42.8%	0.3%	19.4:42.8:28.8
5	187	234	229	155	2,820 μ S	8.3%	1.85%	36.4%	1.1%	30.4:35.7:25.1
6	69	329	409	70	2,460 μ S	92%	1.01%	35.4%	0.3%	17.9:<.1:8.6 ⁱ
7 LoDex10	4	1	1	1	30 μ S	<5%	51.7%	63.6%	40.6%	91.0:<.1:<.1
8	4	1	2	1	53 μ S	<5%	44.6%	49.2%	29.2%	78.2:2.0:<.1 ⁱ
9	8	3	9	1	85 μ S	<5%	48.2%	59.6%	37.2%	53.5:8.1:<.1 ⁱ
10	32	53	47	22	618 μ S	8.8%	43.2%	53.8%	37.2%	87.6:<.1:<.1 ⁱ

^aThe identity of the material and its manufacturer can be obtained by contacting the International Aloe Council, Irving, TX.

^bDetermined at Inchoape Testing Services according to IASC procedure by Inductivity coupled Plasma Atomic Emission Spectroscopy. Data expressed are concentrations in mg per liter of a 0.62 mg/ml solids solutions.

^cConductivity of a 0.62 g~dl solution at ambient laboratory temperature. Test conducted at Inchoape Testing Service.

^dConducted according to the IASC protocol. "E Peak" data is expressed as a percentage of the ARF Standard Sample currently in use at time of testing. Test conducted at Inchoape Testing Service.

^eAssay conducted at Inchoape Testing Service on 500 mg of sample using the published method (Pelley et al. 1993). Values expressed are the % of solids that are alcohol precipitable hexose.

^fAlcohol Precipitable Solids. This is the mass of solid precipitated from the 500 mg sample at Inchoape Testing Service determined by lyophilization at UTMB. Data expressed are percentage of precipitated dry mass versus the 500 mg of powder originally tested.

^gLyophilized, alcohol precipitates were exhaustively dialysed. Data expressed are the percentage of retained material versus the 500 mg of powder originally precipitated.

^hRetained material was hydrolysed in 6N constant boiling HCl for 10 to 20 minutes at 120°C. After neutralization, ratio of mono-saccharides determined by HPLC on a Dionex PA-1 column with a Pulsed Amphoteric Detector. Data expressed are percentages of individual sugars versus the total sugars identified on HPLC.

ⁱUnusual sugars were identified. Sample #6 was 74% amino sugars; sample #8 was 15.5% fructose; sample #9 was 28.1 fructose; sample #10 was 9.7% fructose.

critical representative materials that were acquired and transmitted by a contracted third party. The testing laboratory, Inchoape Testing Service, was the same laboratory that was used by the International Aloe Science Council (IASC) for its Certification Program and was experienced in the collection of evidentiary material in the detection of fraud. Samples were transmitted with regard to proper chain of evidence. All testers were thoroughly blinded as to the identity of the materials. In instances where the UTMB laboratory performed the critical hydrolysis and sugar analyses, the nature of the sample was masked to eliminate any potential bias. Lastly, data from the two analysis streams (Inchoape Testing Service and UTMB) were kept separate until final reports were prepared. As a form of internal quality assurance, one test (phenol sulfuric acid test component of alcohol precipitable hexose assay for total polysaccharide) was performed in common in both the Inchoape Testing Service Laboratory and the UTMB Dept. of Pharmacology and Toxicology. The correlation (R^2 on linear regression) between the results obtained from these two laboratories was 97.9%. All of the materials tested in Study B were of products tested in Study A (although all materials were of different lots, separated by one year from the materials tested in Study A). The data, presented in Table 3, can therefore, be interpreted with a high degree of confidence that

findings were not due to possible technical or systematic error or tampering.

Two newly produced ARF Standard Samples were tested - one of *Aloe barbadensis* gel (Table 3, sample 1) and one of Whole Leaf Extract (Table 3, sample 4). The new ARF Standard Sample of gel had a higher conductivity than those used to generate the data shown in Table 1, but the results were still within the 2σ statistical limit. The alcohol precipitable solids results for the new ARF Gel Standard Sample were identical to the previous samples. Total polysaccharide of the new ARF Gel was lower than the previous sample group (6.22% versus $9.2 \pm 0.7\%$) but it was within the 2σ limit. "E Peak" values for the new ARF Standard Sample of Gel were comparable to those for the current IASC "E Peak" standard. These findings suggest that not only was the new ARF Gel sample representative of the earlier group of ARF Standard Samples but that the Inchoape Testing Service obtained values congruent with earlier findings from the UTMB laboratory.

ARF Whole Leaf Standard Sample was also tested (Table 3). Unfortunately, unlike gel, there is not an extensive data base available for Whole Leaf Extracts. When dissolved to a concentration of 0.62 g per 100 ml, the whole leaf extract did not differ greatly in ionic strength or malic acid content from ARF Standard Sample Gel. The polysaccharide content (0.99%

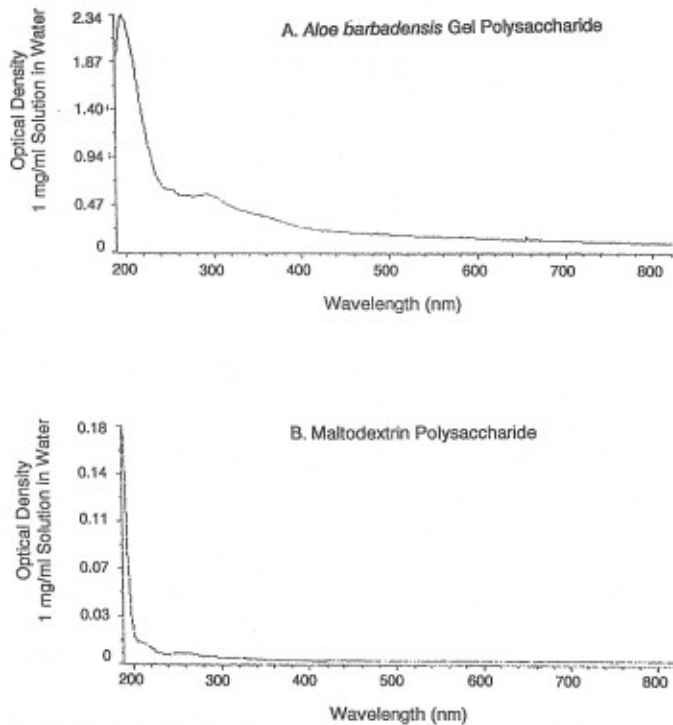


Fig. 2. UV/Visible spectrophotometry of isolated polysaccharides. Panel A represents polysaccharides isolated from ARF93A by exhaustive dialysis followed by precipitation with 80% ethanol. Panel B is isolated from commercial maltodextrin by exhaustive dialysis. Note, that the Y-axis for Panel B is less than one tenth the magnitude of the Y-axis in Panel A.

of total solids) of this material was significantly ($> 3\sigma$) lower than the ARF Standard Sample Gel group in Table 1. However, this polysaccharide value was not different from three previous ARF Standard Samples of Whole Leaf extract prepared over the time period of 1991 to 1993 (data not shown). Therefore, although this Whole Leaf Standard Sample did not differ from *Aloe barbadensis* gel by IASC Certification Parameters, it did differ in polysaccharide content, for which there are no IASC Certification Standards nor Texas A & M University population values available.

As in Study A, the results from powdered materials analyzed in Study B clearly segregated into 3 groups. Samples from two companies (Table 3, samples 2 & 3) were essentially identical to ARF Gel. Samples 5 and 6 were essentially identical to ARF Whole Leaf Extract. Sample 6 was from the same company that manufactured authentic gel (sample 2) and its label clearly identified it as being of Whole Leaf origin. However, the manufacturer of sample 3, whose values were consistent with Whole Leaf Extract, did not identify its extract as being of Whole Leaf origin and the levels of "E Peak" were in the "low" range. Samples 8 and 9 were indistinguishable from authentic commercial Maltodextrin although both of these materials had labeling (200XXX and 200) that unequivocally claimed to be, by IASC Nomenclature, unadulterated freeze-dried "Aloe Vera". Thus, the results obtained by the Incheape Testing Laboratory for samples 2 through 6 were congruent with the materials in Table 2 that

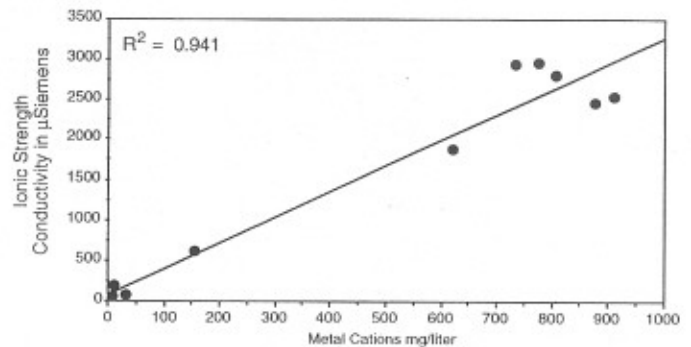


Fig. 3. Relationship between sodium, potassium, calcium, and magnesium ion content and conductivity for commercial materials of defined provenance. The ratios of ions for the commercial materials of defined provenance. The ratios of ions for the different species was determined by simple addition for the values by ICP in Table 3. Ionic strength was determined by conductivity. For the 10 samples of Study B, $R^2=0.941$

were consistent with authentic "Aloe Vera". With the exception of sample 5, there is a reasonable probability that they would pass IASC Certification.

Sample 10 had the greatest degree of similarity to the maltodextrin products but was distinguishable from maltodextrin in the higher levels of Metal Cations, by higher Ionic Strength and by the presence of low levels of "E Peak". In these respects Sample 10 closely approximates the earlier group III materials that were thought to be a mixture of "Aloe Vera" and maltodextrin. The label bore the term "Freeze Dried" which by IASC Nomenclature is assumed to represent "Aloe Vera" without additives (Science and Technical Committee, 1991). The package label had no indication of maltodextrin addition. However, the analytical specification sheet enclosed stipulated that tests were run assuming a "99:1" concentrated product. In the Aloe industry this is generally presumed to represent spray dried product which by the nature of the process contains 50% (w/w) maltodextrin. Therefore, this "Freeze Dried" material probably represents an example of mis-labeling of "spray dried" material as "freeze dried" rather than purposeful adulteration. Whether this was intentional mislabeling or an unintentional mistake cannot be determined. It is interesting to note that the manufacturer of this product also manufactured Sample 5, the material consistent with Whole Leaf but not identified as being of Whole Leaf origin. The low malic acid content of this sample was suggestive of high bacterial levels during processing.

Individual Cations, Total Cations and Conductivity.

Among the samples in Table 3 consistent with "Aloe Vera" there was a certain amount of non-systematic variability in the individual metal cations. This type of variability has been previously noted by Wang and Strong (1995) in their comprehensive population survey of authentic *Aloe barbadensis* gel extracts. They noted that when one ion was in the low end of the normal range, the others tended to be near or above the mean for the population. Thus, the total content of metal cations in the sample tended to be constant. Our samples

confirmed, on a small scale, their observations (Figure 3). The ARF Standards and six samples consistent with authentic "Aloe Vera", clustered on the X axis with total metal cation contents of between 600 and 900 mg/liter. In samples 1-6, the Na⁺ and Mg²⁺ varied over a 5 fold range and K⁺ varied over an almost 4 fold range. This seemingly random variability of individual cations was minimized when total cation content was considered (Figure 3, total cations in the same 6 samples varied by only 30%) (Mean \pm S.E.M., 786 \pm 42 mg/liter; range 621-909 mg/liter).

The lower variance of summated four cation determination was first noted by Wang and Strong in a preliminary report of theirs (1983). In that study, they added the determination of K⁺ and Na⁺ to the IASC Certification parameters (Mg²⁺ and Ca²⁺). Based on this, we subsequently

employed ionic strength, measured as conductivity, to screen samples of commercial "Aloe Vera" (Pelley et al., 1993). Figure 3 demonstrates that, for the 10 samples in Study B, the correlation between Ionic Strength and the content of the 4 metal cations was quite good (94% correlation).

Subsequently, we have learned that some companies in the Aloe industry have been discretely using conductivity for a number of years as a screening test for dilution (Al Davis, oral communication, 1994 IASC Scientific Symposium, Dallas, TX).

Alcohol Precipitable Solids. A test for alcohol precipitable solids, also known as methanol precipitable solids, is still performed the Aloe industry although no standard method has been published nor database defined. Because of a lack of chemical sophistication in the industry, the test is widelybelieved to measure polysaccharide. This test failed to

Table 4. Characteristics of commercial "Aloe Vera" liquid materials.

No	Source ^a	Unfractionated Material			Polysaccharide Isolated by Dialysis					Dialysate	
		Discrepancy in solids ^b	Ionic Strength ^c	Polysaccharide eContent ^d	HPLCe "E Peak"	%Retained material ^f	% Hexose ^g	UV Scan ^h	Giu:Man:Gal Percentage ⁱ	% Hexose ^j	% Reducing Sugar ^k
1	A	16%	2,484 μ S	11.1%	Normal	12.4%	65.2%	0.55	4:90:3	10.7%	13.9%
2	A	-8%	1,799 μ S	5.9%	Normal	10.0%	75.9%	0.19	7:81:4	16.8%	14.7%
3	A	-22%	2,108 μ S	7.9%	Normal	12.4%	50.7%	0.02	18:81:1	16.3%	11.2%
4	R	NP ^l	2,548 μ S	4.5%	Normal	13.1%	66.6%	0.26	18:81:1	10.2%	11.2%
5 ^l	D	16%	773 μ S	2.4%	Low	4.5%	56.3%	0.20	32:51:12	15.6%	N.D.
6 ^l	Q	-10%	1,800 μ S	4.7%	Low	3.4%	68.4%	0.65	62:17:8	14.4%	10.3%
7	Q	42%	1,830 μ S	2.8%	Low	2.1%	71.3%	1.17	69:6:8	16.9%	N.D.
Mean		19%	1,906 μ S	5.6%		8.3%	64.9%	0.43	12:83:2	14.4%	12.3%
S.E.M.		5%	223 μ S	1.2%		1.8%	3.3%	1.15	4 2 1	1.1%	0.9%
8	N	NP	407 μ S	2.6%	Absent	3.3%	38.9%	max250	27:61:3	1.8%	3.0%
							31.8				
9	S	NP	12 μ S	15.2%	Absent	10.9%	74.5%	0.06	73:8:0m	5.5%	2.7%
10	T	-72%	1,538 μ S	3.9%	ABN*	21.9%	27.7%	0.38	68:10:15	3.1%	7.6%
11	T	60%	3,333 μ S	13.9%	ABN*	13.9%	45.5%	2.69	11:79:5	1.6%	5.2%
12	U	-45%	636 μ S	2.3%	Absent	1.7%	61.2%	1.20	53:47:0	18.1%	15.5%
13	U	9%	664 μ S	2.1%	Absent	2.5%	51.7%	1.64	58:42:0	56.2%	15.2%
Mean		47%	1,098 μ S	6.7%		9.0%	49.9%	1.19	48:41:4	14.4%	8.2%
S.E.M.		14%	491 μ S	2.5%		3.3%	6.8%	0.47	10 11 2	8.7%	2.4%

^aSources are as follows: A is a Feedstock manufacturer, D was supplied by a Manufacturer's Representative and is not A. Q is a Broker blending products from Feedstock Manufacturer A, R is a Consumer Product Manufacturer and Sample #7 is a consumer product, C is a Broker, S, T, & U were supplied by Consumer Product Manufacturers.

^bAs describe in Table 2. For determination of means, the absolute value is used.

^cConductivity of a solution with solids content of 0.58 to 0.62 g/dl at ambient laboratory temperature.

^dValues expressed are the % of solids that are alcohol precipitable hexose.

^eAs determined under IASC Conditions. * Extremely abnormal chromatogram

^fLiquids were lyophilized and than dialysed exhaustively until the conductivity of the final dialysate was under 100 μ Siemens. The data are expressed as percentage of mass in dialysate compared to total mass recovered.

^gDetermined by the phenol sulfuric acid assay for total hexose.

^hExtinction coefficient of a 0.1% (1 mg/ml) solution in deionized water.

ⁱRetained material was hydrolysed in 6N constant boiling HCl for 10 to 20 minutes at 120°C. After neutralization, ratio of mono-saccharides determined by HPLC on a Dionex PA-1 column with a Pulsed Amphoteric Detector. Statistical values for commercial materials are form the 4 samples of gel.

^jDetermined by the Neocuprione assay for reducing sugar (Nelson Assay).

^kNP, Not Pertinent. Either there was no label claim for content, or the solids content was discrepant with pure Aloe but there was no mis-labeling.

^lWhole leaf preparation, polysaccharide sugar composition is within normal limits: Glucose, 54 \pm 11%; Mannose, 25 \pm 13%; Galactose, 9 \pm 1%.

^mAbnormally high in fructose, 17/7%.

discriminate between groups of commercial Aloe materials (Table 3, column 10). Aloe materials derived from widely differing sources (gel versus whole leaf) that have polysaccharide contents that differ by 5.7 fold in their polysaccharide content were essentially identical by the alcohol precipitable solids test. Furthermore, although samples 8-10, thought to be fraudulent and/or adulterated materials, had $56.6 \pm 3.2\%$ alcohol precipitable solids, this result was only marginally higher than authentic Aloe.

As Table 1 demonstrates, the alcohol precipitable solids test does measure polysaccharide. If ARF Standard Sample Process A *Aloe barbadensis* gel is treated with 80% alcohol, an average of 35% of the mass of solids is precipitated. Approximately 26% of this precipitate is polysaccharide hexose, the remaining 74% is a complex of divalent metal cations and multivalent organic acids. The additional remaining non-polysaccharide material can be removed by dialysis. Although the data with the ARF Standard Samples is quite uniform, the test is difficult to standardize because the degree of precipitability of the metal cations complexed with the organic acids is a function of both concentration and pH. Therefore, the significance of results using this test can be easily over interpreted and there is the potential for considerable laboratory to laboratory variability in an undocumented test.

The results from Study A showing a relationship between polysaccharide content (alcohol precipitable hexose) and the amount of crude polysaccharide isolated by dialysis and segregation of the data into three groups, were confirmed in Study B (Figure 4). In Study B, the relationship between polysaccharide content and polysaccharide isolation was much better than that observed for samples used in Study A. This could be due to the maltodextrin-containing samples having a significant content of maltodextrin oligosaccharide which were greater than 1-2,000 kD but which were alcohol soluble. Native *Aloe barbadensis* gel contains relatively few oligosaccharides and therefore fractionates in a cleaner fashion

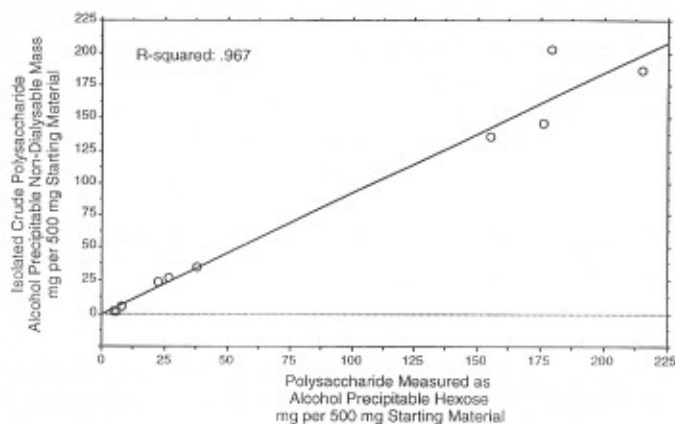


Fig. 4. Correlation between methods used to measure polysaccharides in commercial materials of defined provance. Polysaccharides were measured as alcohol precipitable hexose and yield of crude polysaccharide isolated by alcohol precipitation and dialysis. Data are from the analyses of 500 mg samples of the materials in Table 3.

in the intermediate molecular weight range. Although there was a close relationship between polysaccharide measured as alcohol precipitable hexose and physical isolation of polysaccharide, there were only weak negative correlations between alcohol precipitable solids and alcohol precipitable hexose ($R^2 = 0.078$) or isolated polysaccharide ($R^2 = 0.0082$). Lastly, comparison of the data in Table 3, column 10 and 11 reveals that in the case of gel, 79% of the alcohol precipitable solids "polysaccharide" were, in fact, dialysable. In the case of Group II Aloe material from whole leaf sources, 97% of the alcohol precipitable solids "polysaccharides" were dialysable. Thus, continued commercial reliance on this unpublished method, still widely believed by significant numbers of individuals in the industry to measure polysaccharide, is completely unsupported by the scientific evidence to the contrary.

Analysis of "Aloe Vera" Liquids

The analysis of commercial liquids purported to be "Aloe Vera" is more complex than that of powders. Table 4 gives the results of analyses of 13 liquid samples. The solids content of the samples was determined by lyophilization and compared to that expected from label claims. Their concentrations were adjusted to the solids content of fresh Aloe gel and polysaccharide content was determined on 500 mg of solids. They were then analyzed for their conductivity and "E Peak" content. As in the analyses of Study A commercial powders, there was a group of materials consistent with commercial "Aloe Vera" (Table 4, samples 1-7) and another population (samples 8-13) which greatly differed from the ARF Sample. In the case of liquids, the measurements of polysaccharide content were less valuable in grouping the materials than was correspondence of solids content to label claim, conductivity and HPLC for "E Peak" (malic acid). The data presented below suggest that glycerol and surfactants were preferred to maltodextrin as adulterants in liquids, although small amounts of maltodextrin were detected in some samples.

Four of the seven samples classified as consistent with "Aloe Vera" were relatively easy to identify while the other three were classified with somewhat lower assurance. All three tests based on IASC parameters were useful in discriminating between the groups. The average discrepancy between IASC solids content and label claim for the authentic group was 19% which is well within the limits of variability for the IASC population. The conductivity for samples 1-4 was within 1 σ for the ARF Standard Samples and normal levels of "E Peak" were detected. Lastly, the levels of total polysaccharide were within the limits of the ARF Standard Samples. The classification of samples 5 through 7 was less certain. Their conductivity was somewhat lower than expected and in one case (5) was outside the 2 σ limit. Levels of "E Peak" were below (5 to 10% of ARF Standard Sample) the normal range and the polysaccharide levels were below the 2 σ limit for the ARF Process A Standard Samples.

The residues formed upon drying commercial samples 1-7 were either gum or powder which were indistinguishable from authentic Aloe. In contrast, the physical appearance of the residues formed by the second group varied greatly. Sample 8 totally failed to dry to a powder and yielded a viscous liquid

syrup which consisted of a low molecular weight substance that was hydroxyl rich but not a sugar. Thin layer chromatography (TLC) analysis revealed the presence of massive amounts of glycerol. Sample 9 also dried to a syrup and had the TLC and spectroscopic properties of a nonionic surfactant. This material was most notable for an extremely low ionic strength, completely inconsistent with Aloe. Malic acid was completely absent from these materials. Samples 10 and 11 were similar in their physical properties yielding a macroscopically crystalline material upon drying. Ionic strength was significant but the HPLC profile disclosed a large amount of material absorbing at 205nm which eluded with the solvent front and tailed for a considerable distance downfield. Both of these materials were also distinguished by a large deviation between the solids content expected from label claims (they were concentrates) and the observed solids content. Samples 12 and 13 were abnormal in that their ionic strength was low and "E Peak" (malic acid) was absent. However, it should be cautioned that the quantitative aspects of tests for solids content, ion content, HPLC profile and total polysaccharide content did not give a high degree of confidence for determining whether or not these materials were Aloe.

Polysaccharide analysis suggested that sample 11 had a group I-like (native Aloe gel) polysaccharide because of its low glucose, high mannose, and significant galactose content. In contrast, sample 9 had a polysaccharide sugar composition that was clearly identical to a high fructose maltodextrin. Samples 12 and 13 had polysaccharides suggestive of Aloe adulterated with maltodextrin. Sample 8 and 10 had polysaccharide similar to Whole Leaf Aloe. Thus, in liquids inconsistent with pure Aloe, the wide variety of polysaccharide types mirrored the heterogeneity observed with the other tests. Polysaccharide analysis and simple tests suggested that some materials (Table 4, sample 9) were possibly fraudulent consisting of maltodextrin and surfactants. Other materials such as sample 11, appeared to be authentic Aloe Gel contaminated with large quantities of adulterating chemicals. Still others (samples 12 and 13) may be spray dried Aloe heavily adulterated by dilution with foreign material. Lastly, others (samples 8 and 10) may be Whole Leaf Aloe or extensively processed Aloe Gel heavily adulterated with glycerol or surfactants.

DISCUSSION

For years the Aloe Industry has been crippled by arguments over what is the appropriate "magic molecule" to function as the *sine qua non* for identity as "Aloe". This study utilizes multiparameter analysis of the molecules - polysaccharide, simple sugars, organic acids, and mineral cations - that make up greater than 80% of the solids mass of "Aloe" juice. Thirty-nine samples of commercial materials originating from 21 sources were examined in the present study. The results demonstrated that seventy-one percent of the suppliers were vending material that was not consistent with what it was claimed to be - pure *Aloe barbadensis* extract. Previously, we (Pelley et al. 1993, Waller et al. 1994) suggested that these tests, with modification, could differentiate various populations of commercial materials

alleged to be "Aloe" when used in conjunction with a simple test for polysaccharide. Our results amply illustrate the utility of the International Aloe Science Council (IASC) panel of tests that established parameters for the Certification of Aloe and the database developed at Texas A & M University for these IASC parameters (Wang & Strong, 1995).

It is possible that our estimate of 71% of all feedstock sources of "Aloe" are not *Aloe barbadensis* is an overestimate. The materials in Study A were obtained confidentially from consumer product manufacturers. We have good reason to suppose that they sent us feedstocks that they had reason to hold suspect. Therefore we are probably overestimating the number of "bad" Aloe companies. However, the sheer amount and variety of fraudulent, adulterated and misrepresented "Aloe" raw materials remains staggering. This problem can only be solved by regulatory agencies at the state and national level. The tests we describe herein and referential databases should aid their efforts.

In many cases, because of our pledge of confidentiality in Study A, we do not know the identities of the feedstock manufacturers or brokers involved in this fraud. However, we were able to identify two companies in our initial study that were marketing feedstock materials that were clearly labeled as unadulterated "Aloe" that appeared to consist, almost solely, of maltodextrin. In Study B, we were able to obtain materials from these companies with intact chain of evidence and whose identities are known to the IASC. The Study B materials from these companies gave identical analytical results to Study A - maltodextrin. The case of a third company is more complex. Material with their label, tested in the fall of 1992, yielded one fraudulent (100% maltodextrin) product and one mislabeled/misbranded (Aloe with 50% maltodextrin labeled as 100% Aloe) product. In the fall of 1994, one of their products was without evidence of adulteration and the other (50% Aloe, 50% maltodextrin) is now identified, deep in specification sheets as 99:1 (by implication, 50% Aloe, 50% maltodextrin). We surmise that detailed examination of materials from other sources will yield similar findings. Finally, Study B is based on the report "Project Purity", that was presented by Dr. Nicholas Schmidt at the 1995 Annual meeting of the International Aloe Science Council. The results of that report supports the findings in the present study.

The quality and consistency of any given reagent particularly those derived from natural products, is a major concern to laboratories performing biological research. Early studies from radiation biology using fresh unprocessed Aloe, reproducibly showed significant beneficial effects of the preparation. However, commercial Aloe has consistently failed to provide reproducible results in nearly all biological systems in which it has been tested. One explanation for the variability has traditionally been differences between laboratories and the details of their assay systems. However, in light of the results of the chemical analyses of commercial Aloe presented in this study, the composition and indeed the very identity of a material as Aloe is a major factor which must be considered in future studies of biological activity. Recently (Strickland et al. 1994) we have found that *Aloe barbadensis* gel extracts are efficacious in reversing UVB-induced damage to the skin

immune system in experimental animals. This has an ultimate implication for preventing serious skin cancer in humans. We utilized for those studies ARF Process A Standard Samples (which are not commercially available and do not correspond to any commercial material). We are currently engaged in defining the families of molecules that mediate those biological properties, a task beyond the scope of this discussion. However, we can state that if bacterial proliferation occurs to the point where the organic acids (such as malic acid, i.e. "E Peak") are consumed, biological activity in our system is lost. Of the 18 samples consistent with unadulterated *Aloe barbadensis* extract, only 11 (61% of authentic materials, 28% of all materials) retained markers ("E Peak") consistent with low levels of bacteria prior to commercial processing. Therefore, it is highly likely that approximately 75% of all material vended as "Aloe Vera" is devoid of at least that biological activity. It is probably prudent for scientists studying the biological properties of *Aloe barbadensis* and manufacturers of consumer products to closely consider the true identity of the feedstocks they employ before even beginning to evaluate biological activity.

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