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Physical and chemical characteristics of *Aloe ferox* leaf gel

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Abstract

Aloe ferox leaf gel differs substantially from that of *Aloe vera* but almost no commercially relevant data is available this species. Leaf dimensions, gel yields and gel compositions were studied, based on samples from several natural populations. Glucose is the only free sugar in aloe gel (0.1 to 0.4 mg ml^{-1} in *A. ferox*). Monosaccharides released after hydrolysis show potential for gel fingerprinting and allow for a distinction between *A. ferox* and *A. vera*. The former yields various combinations of glucose and galactose as main monosaccharides, while the latter yields only mannose. Further variation studies are recommended because *A. ferox* appears to have three different gel chemotypes. Conductivity shows species-specific ranges — in *A. ferox* below 3000 μS cm^{-1} in fresh gel and above 3100 μS cm^{-1} in aged gel (corresponding values for *A. vera* were 1670 and 1990 μS cm^{-1}). The level of phenolic (bitter) compounds in *A. ferox* gel can be reduced by treatment with activated charcoal, resulting in a small loss of total dissolved solids. Alcohol precipitable solids and insolubility are useful variables for quality control of gel powder. The methods and data presented are the first steps towards developing quality criteria for *A. ferox* leaf gel.
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Keywords: Free sugars; Gel composition; Gel conductivity; Gel yield; Hydrolyzed sugars; Organic acids; Quality control

1. Introduction

Aloe ferox Mill. (=*Aloe candelabrum* A.Berger), commonly known as the bitter aloe or Cape aloe (also *khala*, *umhlaba*, *bitteraalwyn*) is a polymorphic species indigenous to the Cape coastal region, from Swellendam in the west to the southern parts of KwaZulu-Natal in the east (Reynolds, 1950; Van Wyk and Smith, 1996; Glen and Hardy, 2000). It is a single-stemmed aloe with erect racemes of red, orange, yellow or rarely white flowers and spreading or gracefully curved thorny leaves. Northern forms of the species, previously known as *A. candelabrum*, are morphologically, genetically and chemically within the range of variation of *A. ferox* (Viljoen et al., 1996).

Aloe marlothii A.Berger is a possible alternative source of gel products. This species formed the basis of a pharmaceutical product known as "Natal aloes" that was discontinued in the late 19th century. *Aloe arborescens* Mill., currently under commercial development in Japan and Italy (known as "Japan aloe" or "Kidachi aloe") is another possible alternative source of gel products (Van Wyk et al., 2009).

A. ferox has been used since ancient times as traditional medicine — a San rock painting depicts the plant (Reynolds, 1950) and it has a well-documented history of use as medicine (see Grace, 2011). However, the use of the inner, non-bitter gel as a food supplement is a recent development — no documentation of a food use is found in the literature except the production of jam (preserve) by Cape farmers (Palmer and Pitman, 1972; Fox and Norwood Young, 1982; Palmer, 1985; Rood, 1994). The gel of *Aloe maculata* All. and *Aloe zebrina* Baker, however, is used as famine food in case of an emergency, and the flowers of several species, including *A. ferox*, contain nectar which is eaten by children (Fox and Norwood Young, 1982).

Aloe vera L. is undoubtedly one of the most important medicinal plants of the world. The plant provides the raw materials for a well-researched, well-established, multi-billion dollar industry with an estimated annual turnover exceeding 110 billion US dollars (International Aloe Science Council, 2004). The gel from this species is used mainly in cosmetics and as a tonic drink but there are numerous other uses in the food industry (Grindley and Reynolds, 1986; Reynolds and Dweck, 1999; World Health Organization, 1999; Waller et al., 2004); see

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also Reynolds (2004), Park and Lee (2006), Du Preez (2008), Grace et al. (2008, 2009) and Grace (2011).

In South Africa, the *A. ferox* gel industry has gained momentum since 1994 when the first gel was produced through a patented process in a factory at Albertinia (Botha, 1994; Newton and Vaughan, 1996). Despite the increasing commercial importance of *A. ferox* gel, only one scientific study of the gel components has been published (Mabusela et al., 1990). In view of the large number of *Aloe* species, it is surprising that no research has been done to investigate the commercial potential of other species. In 2007, Standards South Africa (a division of the South African Bureau of Standards) published a South African National Standard for *Aloe* raw materials (Standards South Africa, 2007). The scope of the standard is to specify the "requirements and test methods for *A. ferox* raw material intended for use in consumer products including health, cosmetic, health food, medicinal, veterinary and industrial products".

Gel composition at a species level differs substantially. Available published information shows that the gel composition of *A. vera* (Choi and Chung, 2003; Waller et al., 2004) differs from that of *A. ferox* (Mabusela et al., 1990), and *A. arborescens* (Yagi et al., 1985). These species differ in their acetylated polysaccharides. Nuclear magnetic resonance spectroscopy (NMR) is used as a quality control method for *A. vera* gel but the absence of acetylated compounds in *A. ferox* complicates the application of NMR methods in this species. Thus, quality control methods that are applied to *A. vera* gel cannot be applied with the same success to *A. ferox* gel. The gel polysaccharides are different within these two species, with *A. vera* releasing mannose (Choi and Chung, 2003) after a hydrolysis treatment and *A. ferox* releasing mainly glucose and galactose (Mabusela et al., 1990).

Since almost all published information is only applicable to *A. vera* gel, this study was conducted to explore some chemical and physical characteristics of the leaf parenchyma gel of *A. ferox*. Leaf dimensions, gel fillet yields and gel powder yields were studied in detail. Other parameters investigated included total dissolved solids, free and hydrolysed sugars, conductivity, alcohol precipitable solids and solubility. The expectation was that such data can provide a better understanding of the basic principles underlying gel variability, with possible applications in chemotaxonomy and especially in developing commercial quality control procedures for *A. ferox* gel.

2. Materials and methods

2.1. Materials

Mature leaves were harvested from various individual plants at several different localities throughout most of the natural distribution area of *A. ferox* (with permission from the land owners). Locality details and voucher specimens are listed with the results in Tables 1 to 4.

2.2. Leaf dimensions and gel firmness

Leaf width and leaf thickness measurements were taken at the base of the leaf. A penetrometer was used to determine

Table 1
Leaf dimensions and gel firmness in eight populations of *Aloe ferox* (six leaves from three individual plants were sampled). The values given are averages for six leaves per plant.

Locality and voucher specimens (all in JRAU)	Plants	Leaf weight (g)	Leaf length (mm)	Leaf width (mm)	Leaf thickness (mm)	Gel firmness (kg pressure)
1. Albertinia (S 34° 05.532′; E 21° 38.528′) *O'Brien 60*	1	726.0	471.7	101.7	19.5	10.5
	2	532.7	437.2	106.0	16.2	10.0
	3	628.6	462.7	107.8	17.2	7.1
2. Albertinia (S 34° 08.689′; E 21° 39.309′) *O'Brien 61*	1	523.0	401.7	132.8	10.8	9.6
	2	334.4	323.3	96.8	14.3	6.4
	3	305.0	336.7	88.3	13.5	8.1
3. Seweweekspoort (S 33° 27.480′; E 21° 25.485′) *O'Brien 62*	1	917.7	597.7	119.7	18.7	8.0
	2	950.7	596.7	127.5	22.2	6.9
	3	994.7	534.2	136.3	20.2	6.2
4. Uniondale (S 33° 47.003′; E 23° 29.115′) *O'Brien 63*	1	612.0	480.5	101.5	11.8	8.8
	2	868.2	554.3	100.3	18.3	8.1
	3	469.3	472.5	94.2	11.8	9.3
5. Uniondale (S 33° 34.582′; E 23° 10.390′) *O'Brien 64*	1	1028.2	619.5	109.8	18.7	7.4
	2	800.5	493.7	106.3	18.2	12.0
	3	773.7	519.0	104.2	17.5	11.1
6. Fort Beaufort (S 32° 55.119′; E 26° 28.647′) *O'Brien 65*	1	474.0	449.7	104.0	14.5	7.9
	2	480.8	425.3	90.0	17.0	9.3
	3	569.8	460.4	98.5	14.1	8.4
7. Balfour (S 32° 32.318′; E 26° 41.414′) *O'Brien 66*	1	224.1	406.7	83.7	08.3	5.7
	2	753.9	528.0	132.4	18.4	6.3
	3	333.0	384.8	89.8	11.2	9.4
8. Seymour (S 32° 34.010′; E 26° 44.868′) *O'Brien 67*	1	766.3	550.2	113.2	18.5	9.1
	2	653.0	565.8	121.8	15.0	7.9
	3	956.5	560.5	112.5	20.3	7.6
Mean value		653.1	484.7	107.5	16.1	8.3

the firmness of each individual *A. ferox* gel fillet. A probe of 11 mm in diameter was pushed into the gel from the middle portion of each leaf to a depth of 8 mm. The values recorded were an average of three repetitions and the unit of measurement is kg pressure.

Table 2
Individual leaf weights, gel fillet weights, gel liquid weights and gel yields (as fillet or liquid) for eight *Aloe ferox* plants (six leaves per plant) from the same eight localities listed in Table 1.

Locality and plant number	Leaf number	Leaf weight (g)	Gel fillet weight (g)	Gel liquid weight (g) (pooled value of six leaves)	Gel fillet yield (%)	Gel liquid yield (%)
1/1	1	496.3	200.6	1067.6	40.4	88.9
	2	523.9	232.0		44.3	
	3	440.2	172.0		39.1	
	4	454.3	232.7		51.2	
	5	464.8	205.8		44.3	
	6	464.8	158.4		34.1	
2/1	1	1057.8	515.9	1570.3	48.8	73.9
	2	698.4	328.7		47.1	
	3	703.9	350.3		49.8	
	4	616.9	229.3		37.2	
	5	795.9	361.9		45.5	
	6	754.9	337.1		44.7	
3/1	1	176.7	71.9	426.0	40.7	76.6
	2	266.9	110.9		41.6	
	3	179.5	76.7		42.7	
	4	220.8	108.6		49.2	
	5	237.4	79.2		33.4	
	6	263.2	108.9		41.4	
4/1	1	1315.5	714.8	1359.3	54.3	43.9
	2	1305.9	698.1		53.5	
	3	854.0	379.4		44.4	
	4	1099.3	580.9		52.8	
	5	894.8	456.8		51.1	
	6	699.5	263.0		37.6	
5/1	1	557.2	264.3	903.8	47.4	56.8
	2	655.4	288.5		44.0	
	3	694.1	313.6		45.2	
	4	658.4	269.2		40.9	
	5	656.3	286.1		43.6	
	6	450.4	160.0		35.5	
6/1	1	783.1	400.9	1310.6	51.2	59.7
	2	737.4	397.4		53.9	
	3	764.7	361.6		47.3	
	4	752.9	387.5		51.5	
	5	565.2	283.9		50.2	
	6	752.9	364.9		48.5	
7/1	1	554.7	205.8	1028.8	37.1	75.5
	2	555.5	247.1		44.5	
	3	495.9	210.7		42.5	
	4	553.5	253.1		45.7	
	5	522.1	245.0		46.9	
	6	466.4	201.3		43.2	
8/1	1	846.2	423.8	2252.6	50.1	81.1
	2	968.9	492.4		50.8	
	3	845.3	414.3		49.0	
	4	925.4	483.7		52.3	
	5	878.6	458.3		52.2	
	6	1037.4	503.9		48.6	

2.3. Hand filleting and gel yields

Harvested leaves were manually filleted in the laboratory on a stainless steel surface. Whole leaves were thoroughly washed and scrubbed to remove mud and bitter exudates before weighing. The sides, base and tip of each leaf were removed, and the inner leaf portion cut longitudinally into strips. The gel parenchyma was then cut away from the rind with a sharp knife. Gel fillet weights were recorded for each leaf. All fillets were then liquidised and filtered through Whatman No. 4 filter paper, applying vacuum until all liquid was removed. The gel liquid recovered was weighed. Gel fillet yield and gel liquid yield were calculated using the following equations:

$$Gel\,fillet\,yield\,(\%) = \frac{Weight\,of\,gel\,fillet}{Weight\,of\,leaf} \times 100$$

$$Gel\,liquid\,yield\,(\%) = \frac{Weight\,of\,gel\,liquid}{Weight\,of\,gel\,fillet} \times 100.$$

2.4. Activated charcoal treatment

For some applications, gel samples were de-bittered using 10 g of granular activated charcoal per 250 ml liquid gel. The suspension was left at 4 °C for 2 h (with periodic stirring) after which it was passed through Whatman No. 1 filter paper to remove the coarse granules of charcoal and then through Celite Filteraide to ensure complete removal of the charcoal.

2.5. Freeze-drying of aloe gel

A bench-top Virtis freeze-dryer (8 l) was used for the drying of gel samples. Gel extracted from the leaves was loaded in 250 ml volumes onto the dryer and dried at a low temperature (−55 °C) under a high vacuum (50 mTorr) for a cycle of 36 h. Samples were 'shelled' using liquid nitrogen to increase the drying surface area and thus decrease the total drying time. The weight of the liquid gel (250 ml) and resulting gel powder was recorded for further yield determination. The gel powder yield was calculated using the equation below.

$$Gel\,powder\,yield\,(\%) = \frac{Weight\,of\,gel\,powder\,obtained\,from\,250\,ml\,gel}{Weight\,of\,250\,\,ml\,gel} \times 100$$

2.6. Hydrolysis of aloe gel

Freeze-dried, de-bittered aloe gel (20 mg) was suspended in 2 ml of a 2 M trifluoroacetic acid (TFA) aqueous solution and heated at 120 °C for 2.5 h. Vials were shaken at 15 min intervals to stop residues from sticking to the bottom of the vials. Once hydrolysed, samples were taken to dryness under vacuum with the addition of 1 ml methanol to accelerate the drying process.

Table 3
Gel powder yield, total dissolved solids (TDS, before and after treatment with activated charcoal to remove phenolic compounds, as % Brix), free glucose level (as mg ml^{-1}), monosaccharides released by hydrolysis (as % of total sugars) and conductivity (in fresh gel and gel left at room temperature for three days, as μS cm^{-1}) in nine *Aloe ferox* gel samples. Some comparative data for *Aloe vera* gel is presented in the last row. nd = not determined.

Locality and voucher specimen (all in JRAU)	Plants	Gel powder yield (%)	TDS (% Brix) before and after charcoal treatment		Free glucose (mg ml^{-1})	Monosaccharides released by hydrolysis (% of total sugars)				Conductivity (μS cm^{-1})	
						Gal	Glu	Man	Xyl	Fresh	Old
Aliwal North *O'Brien 69*	1	0.55	2.0	1.9	0.1	42	58	–	–	2890	3410
Albertinia (Brakkloof) No voucher	1	0.46	1.7	1.6	0.1	–	100	–	–	2760	3190
Albertinia (Middeldrift) No voucher	1	0.21	1.5	1.4	0.2	–	100	–	tr	2702	3325
Albertinia (Vinklaagte) No voucher	1	0.16	1.5	1.4	0.4	–	100	–	tr	2780	3395
Fort Beaufort *O'Brien 60*	1A	nd	nd	nd	nd	33.5	66.5	–	–	nd	nd
	1C	nd	nd	nd	nd	31.5	68.5	–	tr	nd	nd
	1E	nd	nd	nd	nd	–	100	–	–	nd	nd
Uniondale *O'Brien 63*	1A	nd	nd	nd	nd	–	100	–	–	nd	nd
	1C	nd	nd	nd	nd	49.7	50.3	–	–	nd	nd
	1E	nd	nd	nd	nd	48.2	51.8	–	–	nd	nd
Aloe vera (ex hort.) *O'Brien 51*	1	0.60	1.8	1.6	3.3	–	–	100	–	1670	1990

2.7. Chromatographic analysis of free and hydrolyzed sugars

Monosaccharides present in aloe gel or in hydrolyzed gel were analysed directly by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) or as alditol acetate derivatives by gas chromatography (GC).

Table 4
Alcohol precipitable solids (APS) and insolubility percentages obtained for gel samples from eight populations of *Aloe ferox* as listed in Table 1 (six leaves from three individual plants were sampled) and a single sample of *A. vera* for comparison.

Population and plants	Average APS (%)	Average insolubility (%)
1A	52.3	25.5
1C	37.5	3.5
1E	33.2	3.0
2A	25.2	7.7
2C	26.5	8.0
2E	27.8	4.7
3A	48.8	7.0
3C	19.5	7.0
3E	27.7	6.0
4A	38.8	4.0
4C	18.8	4.7
4E	59.5	23.0
5A	39.6	8.0
5C	22.2	3.3
5E	37.0	4.5
6A	21.0	5.0
6C	31.6	5.0
6E	23.0	8.0
7A	36.6	4.0
7C	24.8	3.7
7E	20.0	3.5
8A	23.1	2.0
8C	33.5	12.0
8E	27.6	7.1
Aloe vera (ex hort.) *O'Brien 51*	34.2	20.2

2.7.1. Thin-layer chromatography (TLC)

Various solvents systems were used for TLC on silica gel plates, of which butanol:acetic acid:diethyl ether:water in a ratio of 9:6:3:1 (Harborne, 1988) gave the best results. Plates were developed twice to allow for better separation of poorly separating sugars (they were taken from the solvent, dried and then redeveloped to the original solvent front level). The sugars were detected by spraying with aniline phthalate dip or with chromic acid. Dried plates were heated at 110 °C for approximately 20 min or until a colour change occurred. Spot colours and R_f values were compared to those of reference standards. Freeze-dried aloe gel samples were reconstituted in distilled water to form a 1% solution (w/v). This dissolved sample was passed through a Cameo 0.22 mm nylon filter after which 5 μl was spotted onto the TLC plates and treated and developed in the same manner as the standards.

2.7.2. High-performance liquid chromatography (HPLC)

A Shimadzu LC-6AD HPLC system equipped with a Waters differential refractometer R401 was used. Fresh aloe gel was passed through a Cameo 0.22 mm nylon filter and 20 μl injected directly into the system. The separation was performed on a Hamilton HC-75 H^+ form (305 mm × 7.8 mm) column specifically designed for the simultaneous detection of sugars and organic acids. Conditions of analysis were: 0.01 N H_2SO_4 as solvent with a flow rate of 0.6 ml min^{-1} at an ambient temperature. Pure monosaccharides (8 mg ml^{-1}) were used as external standards. Peaks generated from the aloe gel were identified by comparison of their retention times. Concentrations of monosaccharides were calculated using peak area.

2.7.3. Gas chromatography (GC)

Derivatives of the free sugars were prepared according to the method of Hoebler et al. (1989). Freeze-dried gel was reconstituted in a 1% solution (w/v) with distilled water, after which

2 ml of ethanol was added. The solution was centrifuged, the supernatant decanted, taken to dryness and then reconstituted with 100 μl distilled water. This sample was further processed for GC analysis: 2 ml of a sodium borohydride solution in dimethyl sulfoxide (2:100, w/v) was added to 0.1 ml of sample. The mixture was stirred for 1.5 h at 40 °C. Glacial acetic acid (0.2 ml) was added and the test tubes placed on ice. Once cooled, acetylation was achieved by the addition of 4 ml acetic anhydride and 0.4 ml 1-methylimidazole. The solution was stirred and allowed to stand for 10 min at room temperature. To decompose any excess acetic anhydride, 20 ml of distilled water was added and the tubes again cooled on ice. When cold, 8 ml of dichloromethane was added and the mixture shaken and separated in a separation funnel. The lower phase was removed and washed a further three times using 20 ml of distilled water. The dichloromethane extract was concentrated under vacuum and the resultant alditol acetates were taken up in 1 ml of dichloromethane (CH_2Cl_2) and placed in a freezer 30 min prior to GC analysis. Alditol acetates were analysed on a 30 m × 0.25 mm capillary column DB 100 (J & W Scientific, Inc.) on a Shimadzu Class GC-17A gas chromatograph with a flame ionisation detector (FID). Helium was used as a carrier gas at a flow rate of 4 ml min^{-1}. The column temperature was set at a constant 220 °C, the injector port at 270 °C and the detector at 250 °C. A 1 μl sample in dichloromethane was analysed for a total run time of 25 min. Peak retention times of gel samples were compared to those of monosaccharide standards derivatised as their alditol acetates, following the same procedure.

2.8. Total dissolved solids (TDS)

To determine the effect of the activated charcoal treatment, gel was placed on the stage of a hand-held refractometer, which measures the total dissolved solids (TDS) expressed as percentage Brix (% Brix). Readings were taken before and after treatment of aloe gel with activated charcoal.

2.9. Conductivity

Measurements were taken with a Crison Conductimeter 525. The instrument was calibrated according to the manufacturer's specifications and readings were expressed in μS cm^{-1}. *Aloe* gels were analysed fresh and then again after a three-day storage at room temperature.

2.10. Alcohol precipitable solids and solubility

These two variables were studied by dissolving freeze-dried aloe gel (20 mg) into 2 ml distilled water. Samples were stirred at room temperature for 15 min to obtain a maximum solution of freeze-dried gel powder. Thereafter 6 ml of ethanol (75%) was added and the solution centrifuged for 10 min. The bulk of the upper soluble water phase was discarded. The insoluble precipitate was then taken to complete dryness by freeze-drying. This procedure was repeated three times to obtain an average. The precipitate was weighed and the APS was expressed as a percentage using the following equation:

$$APS\,(\%) = \frac{Weight\,of\,precipitate}{Original\,gel\,powder\,weight\,(20\ \mathrm{mg})} \times 100.$$

The solubility (or actually the insolubility) of the gel was determined by reconstituting the APS fraction (obtained above) in 0.5 ml distilled water. Reconstituted samples were stirred for 15 min. The water layer was decanted and the precipitate remaining was dried (freeze-dried) and weighed. The procedure was repeated three times to get an average. The insoluble fractions were expressed as a percentage of the original gel powder weight in the following equation:

$$Insolubility\,(\%) = \frac{Weight\,of\,insoluble\,precipitate}{Original\,gel\,powder\,weight\,(20\ mg)} \times 100.$$

3. Results and discussion

3.1. Leaf dimensions and gel firmness

The leaf dimensions of 24 *A. ferox* plants from eight populations representing most of the natural distribution area of the species are presented in Table 1. The mean leaf weight is about 0.65 kg, the mean leaf length nearly 485 mm, the mean leaf width almost 108 mm and the mean leaf thickness just over 16 mm. The exceptional variability is noteworthy, probably reflecting a combination of genetic and phenotypic effects. This is in sharp contrast to the remarkable uniformity in commercial *A. vera*, which represents a single clone (an ancient cultigen) and hence almost no variability except those that can be ascribed to environmental effects during cultivation. The results presented in Tables 1 and 2 allowed us to recommend that *A. ferox* leaves should not be harvested if they are less than 30 cm long (Standards South Africa, 2007). The gel firmness and gel yield of small leaves tend to be rather low. Furthermore, smaller leaves are likely to be immature, indicating that they have been harvested in the upper part of the rosette, which should always be left for the plant to recover.

Gels are relatively firm in *A. ferox* leaves, with firmness readings ranging between 6 kg and 12 kg. Firmness is important, especially for hand filleting, because gel cannot be effectively removed if the leaves are too flaccid.

3.2. Gel and gel powder yields

Table 2 shows the individual leaf weights and gel fillet weights for eight plants of *A. ferox* (each with six leaves sampled), as well as the gel liquid weight for all six leaves removed from each *A. ferox* plant. Leaf weights collected from different *A. ferox* populations are very variable (±177 to 1316 g per leaf). Using the hand filleting technique, the average yield of gel fillet per leaf (w/w) is approximately 50% but the actual values vary greatly. For a relatively small leaf weighing only 177 g, the weight of the gel fillet that is removed is 72 g (41% gel fillet yield). For a very large leaf weighing 1316 g, a gel fillet weight

of 715 g is obtained (54% gel fillet yield). In general, small leaves have proportionally lower gel yields than large leaves. When considering gel liquid yields (Table 2), the overall recovery of gel liquid from gel fillet (w/w) was high (up to 89% gel liquid recovery). This is strongly influenced by the water content of the gel. Cellular particles removed by filtering account for a relatively small fraction (sometimes <22%) of the liquidised gel fraction but the extreme variability is again noteworthy. It is important to note that polysaccharides have a tendency to bond fairly strongly to the cellulose in filter paper. A glass fibre filter (Diallo et al., 2003) or centrifugation is therefore recommended for future work.

The percentage gel powder yields obtained in this study are found in Table 3. Gel powder yields range from 0.16 to 1.05%. Gel powder yields obtained after freeze-drying vary greatly between gel batches from different populations (0.16 to 0.55%). These populations were wild harvested and undoubtedly differed in the available soil water and thus the water content of the leaves. The yield of gel powder per leaf may well be fairly uniform but when expressed as a percentage of wet gel weight the yield figures are very variable.

3.3. Total dissolved solids (TDS)

Total dissolved solids (TDS) decreased in gel treated with activated charcoal (Table 3). This is an indication that some solids are lost during the treatment. After industrial filtration of *A. vera* gel, the mixture of charcoal and celite that remain behind may have a hexose content of up to 37% (Waller et al., 2004). The decline in solids using the activated charcoal method developed in this study showed minimal removal of solids with an average decrease of only 0.1 to 0.6% Brix. This is important, as sugars and other solids are considered to be important components of the gel.

3.4. Free and hydrolysed sugars

All three of the chromatographic techniques (TLC, HPLC and GC) confirmed that glucose is the only free sugar found in the gel of *A. ferox* (and several other species — see O'Brien, 2005). The presence of glucose as practically the only free monosaccharide in *A. vera* gel (Christopher and Holtum, 1996; Femenia et al., 2003) is confirmed here. Glucose levels in *A. ferox* gel varied from 0.1 to 0.4 mg ml^{-1}.

All samples were successfully hydrolysed after treatment at 120 °C for 2.5 h. Glucose was found in the hydrolysed gel of all species investigated (O'Brien, 2005) except *A. vera*, which yielded only mannose. Other monosaccharides released after hydrolysis included arabinose, galactose and xylose (O'Brien, 2005), with galactose so far found only in *A. ferox* and *A. cooperi* Baker. In the *A. ferox* populations investigated, galactose is either present or absent (Table 3). It seems that there are three gel chemotypes in this species, irrespective of geographical location: glucose only, galactose–glucose in ratio of 1:1 and galactose–glucose in a ratio of 1:2. Galactose appears to have potential as a diagnostic fingerprint for *A. ferox* gels in the same way as mannose is used in *A. vera*. However, this idea needs further investigation. Xylose appears to be a minor sugar released by hydrolysis, with only trace amounts sporadically present in *A. ferox*. It is important to note that pentoses and 6-deoxyhexoses degrade at a higher rate than hexoses under acidic conditions and high temperature (Aspinall, 1983), so that our results (aimed at chemical markers for quality control) do not reflect the actual composition of hydrolysable sugars. Mabusela et al. (1990) have shown that water extracts of *A. ferox* leaf gel contain arabinose and rhamnose.

3.5. Gel conductivity

An increase in conductivity is associated with the prolonged storage of *A. ferox* gel at room temperature (Table 3). A possible explanation for this relationship is that glucose can be converted into lactic acid which can result in an increase of free ions or conductivity within decaying aloe gels. The conversion of malic acid to lactic acid deserves further study, taking into account the daily fluctuations of malic acid (Christopher and Holtum, 1996; O'Brien, 2005). Levels of conductivity in *Aloe* gels appear to be species specific. The conductivity of *A. ferox* gel is around 3000 µS cm^{-1} (Table 3), *A. speciosa* Baker above 3000 µS cm^{-1} (O'Brien, 2005) and *A. vera* around 2000 µS cm^{-1} (O'Brien, 2005). Conductivity appears to be a good indicator of gel freshness and may have practical value in the quality control of *A. ferox* gel.

3.6. Alcohol precipitable solids and solubility

The percentages of alcohol precipitable solids (APS) and insolubility of 24 gel samples from eight populations of *A. ferox* (and one sample of *A. vera*) are shown in Table 4. The values for APS range from 18.8 to 59.5%, with an average of about 32%, while the insolubility varies from 2.0 to 25.5% (average about 7.1%). Despite this extreme variability, APS (together with insolubility) is a useful measure for quality control of gel powders and have practical implications in product formulation. Suppliers link the biological activity of aloe gel with the APS weight of the gel because the APS value is assumed to be a direct indication of the amount of polysaccharides within the gel. As a result, the method described here was taken up in the South African National Standard for *Aloe* raw materials (Standards South Africa, 2007). Alcohol precipitable solids are nevertheless a crude analytical parameter, as organic acids, metal ions and glycoproteins are also included in the precipitate, together with polysaccharides. Thus, APS determination is directly linked to mineral content and gel solubility. In the South African aloe industry, a unique second process is used in combination with alcohol precipitation (Botha, 1994). In this patented process, the fibrous pulp that remains after liquidised aloe gel has been filtered off, is repeatedly washed to remove all bitter substances, after which it is treated with sodium citrate. Water is added and the mixture is heated. The sodium citrate causes the bonds between polysaccharides and calcium to be broken. The mixture is then filtered, and the liquid fraction, which contains the calcium-free polysaccharides, is known as aloe 'jelly'. This 'jelly' forms the basis for *A. ferox* health drinks in South Africa. The quantitative and

qualitative relationships between APS and 'jelly' polysaccharides in *A. ferox* gel deserve further study. When pectic polysaccharides are present, an HPLC method which can simultaneously measure the presence of both neutral and acidic monosaccharides in hydrolyzates (Dai et al., 2010) would be ideal for quality control purposes.

4. Conclusions

Leaf dimensions are variable between plants from the same population as well as from population to population. The leaves of *A. ferox* are firm, allowing for easy hand filleting. Larger, heavier leaves generally result in larger gel fillets using manual filleting techniques. Gel liquid yields vary considerably within populations as well between populations, much of which can probably be ascribed to varying climatic conditions (especially rainfall and humidity which in turn determine the water content of the leaves). Gel yields are very variable and often different to what would be expected from a superficial inspection of leaf dimensions (without consideration of the relative thickness of the rind and parenchyma). Gel powder yields are exceptionally variable and probably strongly influenced by the water content of the leaves at the time of harvest. Treatment with activated charcoal (to remove phenolic compounds) may result in some loss of solids from the gel.

It can be concluded that considerable quantitative variation is found in monosaccharides released after hydrolysis but that the qualitative pattern is surprisingly invariable. Individual plants of *A. ferox* (regardless of provenance) appear to belong to one of three gel chemotypes (glucose only or galactose–glucose in a ratio of 1:1 or 1:2). This interesting result should be explored in more detail, based on a wider sample. In contrast, *A. vera* gel appears to be invariable and yield only mannose after hydrolysis.

This study has confirmed that the age and storage conditions of aloe gels may influence their conductivity. An increase in gel conductivity may prove to be a useful quality control method for the local gel industry. Fresh gel of *A. ferox* has a conductivity between 2702 and 2890 $\mu S\ cm^{-1}$, while the older gel samples are well above 3190 $\mu S\ cm^{-1}$. Fresh *A. vera* gel has a conductivity of well below 2000 $\mu S\ cm^{-1}$. It will be useful to measure larger numbers of gel samples to get a better idea of the total range of variation in this character so that definite criteria can be established for conductivity as a quality control parameter for *A. ferox*. Alcohol precipitable solids and insolubility show much variation in *A. ferox*, perhaps reflecting genetic differences between plants and populations, in addition to environmental effects.

The data presented here highlights important physical and chemical differences between the leaves and leaf gel of *A. ferox* and those of other species (notably *A. vera*). Despite considerable variation, several parameters deserve further study and more thorough evaluation as potential quality control variables for *A. ferox* leaf gel.

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