Striking differences in Aloe vera gel carbohydrate composition, molecular weight and particle size distributions following processing will not be addressed by dietary supplement GMPs

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ABSTRACT

Background Aloe vera gel (AVG) is a popular dietary supplement ingredient. The International Aloe Science Council (IASC) standards to confirm identity and quality of aloe products are, respectively, polysaccharide acetylation patterns and organic acid profiles. Certain polysaccharides contribute to biological activities of numerous medicinal plants, including aloe vera (AV). The quantity and quality of polysaccharides in AV supplements thus must be better understood. **Objective** Determine the effect of processing methods on AVG carbohydrate composition (CC), molecular weight (MW) and particle size (PS) distributions. **Methods** 3 leaves from plants grown commercially were used to prepare native gel (G) and four blended samples: not filtered (NF), filtered (F), not filtered/ freeze dried (NF/D), filtered/freeze dried (F/D). Also analyzed were two commercial AVG powders from IASC-certified vendors (C1, C2). CC was determined for all samples by HPAEC. Additional tests were performed on all samples except G: MW distributions by HPLC-SEC; PS distributions using a Microtrac® PS Analyzer. **Results *CC*:** *1)* *laboratory* There was variability in major sugar content between leaves: NF samples from leaf 2 contained 45.02% glucose and 38.21% mannose; samples from leaf 3 contained 28.9% glucose and 55.2% mannose. 24% of G was glucuronic acid; no other laboratory preparation contained this sugar. Blending impacted CC: compared with NF, G contained substantially more glucuronic acid, arabinose, rhamnose, and fucose and less mannose and glucose. Fructose and glucosamine, present in small quantities in NF, were not detected in G. Filtering impacted CC: compared with NF, F contained more mannose and less glucose and galactose. Filtering removed xylose, fucose and rhamnose. Freeze drying impacted CC: increasing the percentage of glucose and decreasing the percentage of mannose in filtered and not filtered samples. 2) *commercial* Compared with C2, C1 contained substantially more glucuronic acid, mannose, galacturonic acid and galactose and less glucose. ***MW*:** 1) *laboratory* Filtering did not impact MW distributions. MW distributions of not filtered samples were affected by freeze drying: freeze dried samples contained a higher percentage of >10,000 - <100,000 MW polysaccharides and a lower percentage of <10,000 MW polysaccharides. 2) *commercial* C1 polysaccharides were significantly higher MW (C1: 23.3% >800,000; C2: 40.1% <10,000). ***PS*:** 1) *laboratory* Filtration substantially reduced sample particle sizes. Freeze drying reduced the particle size of not filtered samples. For filtered samples, freeze drying virtually eliminated 1.94-11 µ particles and substantially increased the percentage of 249-592 µ particles, substantially decreased 592-995.5 µ particles and eliminated 995-2000 µ particles. 2) *commercial* C1 had a higher percentage of large particles. **Conclusions** We report that processing significantly impacts AVG carbohydrate composition, molecular weight distribution and particle size distribution. These changes—which likely affect product quality and efficacy—suggest that dietary supplement GMPs may not adequately address these issues.

METHODS

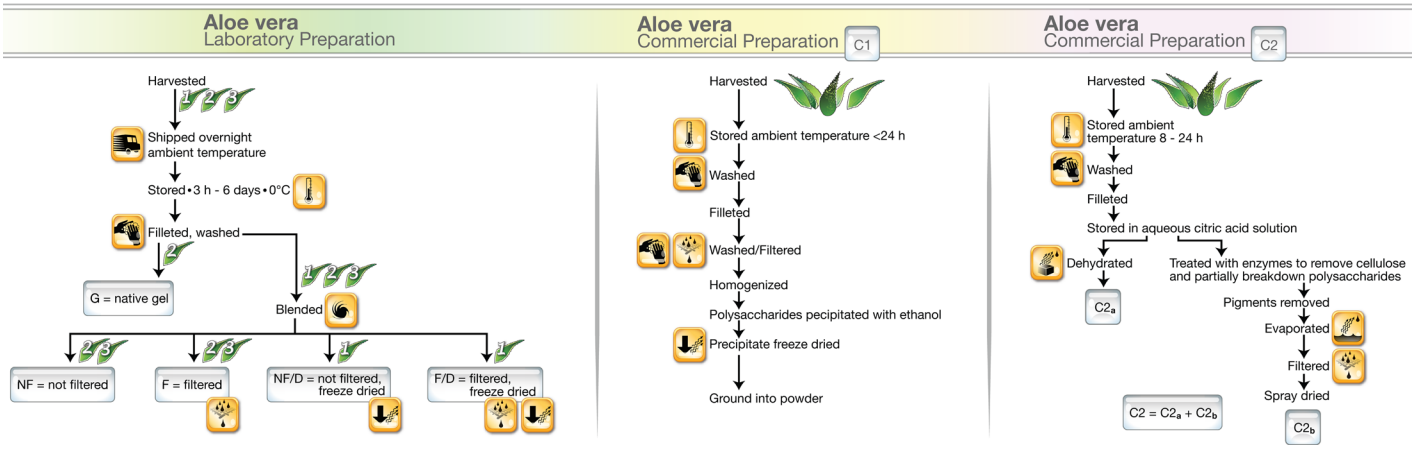
AVG processing The steps for preparation of commercial and laboratory AVG samples are outlined in Figure 1. Laboratory Approximately 2´ leaves from plants grown commercially were shipped overnight. 3 h after receipt, leaf 1 (samples F/D and NF/D) was processed. Leaves 2 & 3 (samples G, NF and F) were stored in a refrigerator for 6 days before processing. Processing: filets were removed from leaves with a sterilized knife, washed for 30 min in a di$H_2$0 bath, and all samples except G were blended at the highest speed in a Waring Commercial blender for 2 min. Half of the blended gel was filtered through three layers of cheesecloth and stored at 0° C. Remaining samples were freeze dried for 6 days in a Labconco FreeZone6 freeze dryer at -22° C, hand milled through a 40-mesh sieve and stored at 0° C in sterile 50 ml Falcon tubes.

Preparation of samples for analyses Triplicate samples of C1, C2, NF/D and F/D were weighed, hydrated in deionized water and placed on an orbital shaker at 200 RPM for 90 min. Duplicate samples of F, NF and G were weighed.

Carbohydrate Composition Triplicate aliquots of above samples were subjected to hydrolysis in 2M trifluoroacetic acid at 120° C for 1.5 h in 16 x 125 mm glass borosilicate test tubes and freeze dried at -22° C for 4 h. Samples were reconstituted using 2-deoxy-D-galactose as an internal standard and filtered through Whatman Mini-uniprep™ 0.2um polypropylene filters into HPLC vials. CC was determined using high performance anion exchange chromatography (HPAEC) (Dionex600) with a CarboPac™ PA10 guard column (4 x 50mm) coupled with a CarboPac™ PA10 analytical column (4 x 250 mm) (CarboPac™ 10 detects: fucose, galactosamine, rhamnose, arabinose, glucosamine, galactose, glucose, N-acetyl-glucosamine, xylose, mannose and fructose). Galacturonic and glucuronic acids were determined using a Dionex ICS2500 system with a CarboPac™ PA20 guard column (3 x 30mm) coupled with a CarboPac™ PA20 analytical column (3 x 150 mm). Monosaccharides were separated with a sodium hydroxide gradient (DX600) and sodium acetate/sodium hydroxide gradients (ICS2500). CCs for each sample were calculated using calibration plots and area ratios of saccharides, an adaptation of a technique published by Eberendu et al.[1]

Molecular Weight MW of triplicate samples was determined using high performance liquid chromatography size exclusion (HPLC-SEC) (Shimadzu Corporation) with a GFC-4000 guard column coupled with a BioSep-SEC-S 4000 (300 x 7.8 mm 5 micron) analytical column.

Particle Size Determination PS of triplicate samples was assessed using a Microtrac S3500 Particle Size Analyzer.



RESULTS

There were striking differences in glucose and mannose concentrations between AV leaves (Figure 2). Laboratory and commercial AVG preparation procedures had significant effects on CC (Figures 3a-3b, Table 1, p. 3), and MW (Figures 4a-4b, p. 4) and particle size distributions (Figures 5a-5b, pp. 4-5). Note: see Abbreviations Key on p. 5.

DISCUSSION

Discrepancies in the CC of AVGs have been attributed to seasonal change and/or geographic affects.[2] This is the first study to examine the stepwise effects of blending, filtering and freeze drying of AVG on CC and MW and particle size distributions. We found notable differences in the AVG glucose and mannose concentrations between two leaves harvested at the same time and processed under the same conditions.

(continued on p. 5)

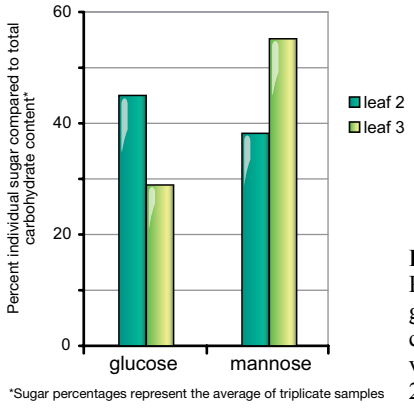
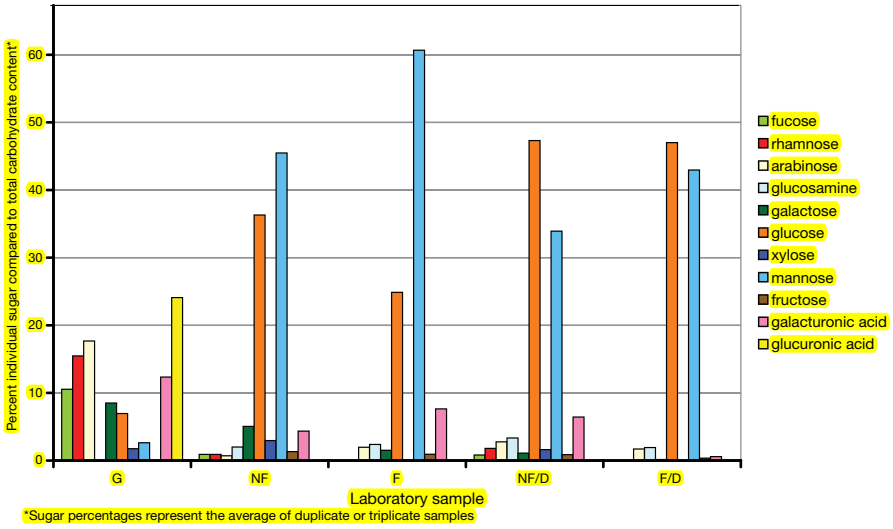


Figure 2. Percent (w/w) glucose and mannose concentrations in aloe vera gel from leaves 2 & 3 (NF)

Table 1.
Percent (w/w) glucose and mannose concentrations in aloe vera gel from leaves 2 & 3 (NF)

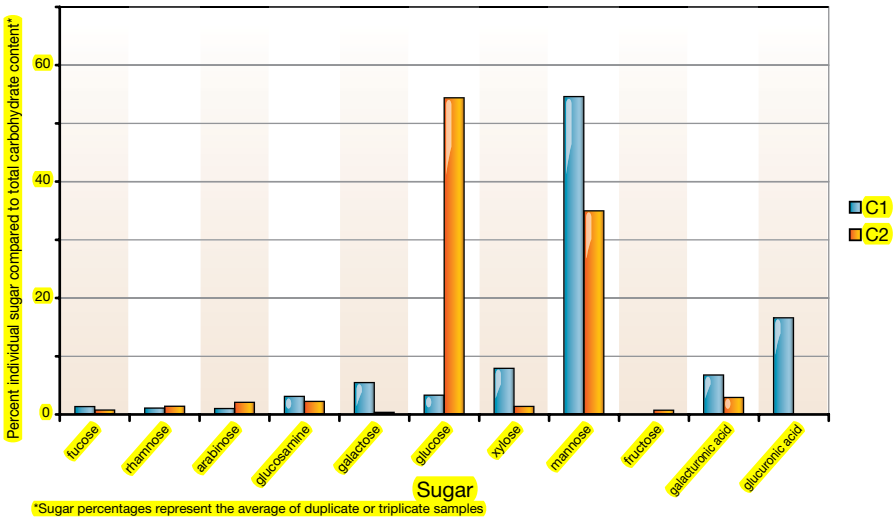
Process	fucose	rhamnose	arabinose	glucosamine	galactose	glucose	xylose	mannose	fructose	galacturonic acid	glucuronic acid
Laboratory											
blending of native gel (NF compared with G)	-0.731	-1.080	-1.243	+0.021	-0.547	-0.115	-0.089	+0.286	+0.014	-0.824	-1.700
filtering of blended gel (F compared with NF)	-0.009	-0.010	+0.001	-0.001	-0.040	-0.160	-0.031	+0.040	-0.006	+0.019	no change
freeze drying of not filtered, blended gel (NF/D compared with NF)	+1.297	+2.863	+4.399	+5.316	+1.704	+75.080	+2.549	+53.623	+1.388	+10.222	no change
freeze drying of filtered, blended gel (F/D compared with F)	no change	no change	+4.028	+4.536	+0.357	+110.883	no change	+100.989	+0.849	+13.832	no change
Commercial											
alcohol precipitation (C1) compared to spray dried/dehydration (C2)	+0.375	-1.327	-2.906	-0.356	+6.897	-110.582	+8.204	+1.838	-1.264	+3.885	+22.881

Figure 3a.
Percent (w/w) sugar content of laboratory aloe samples



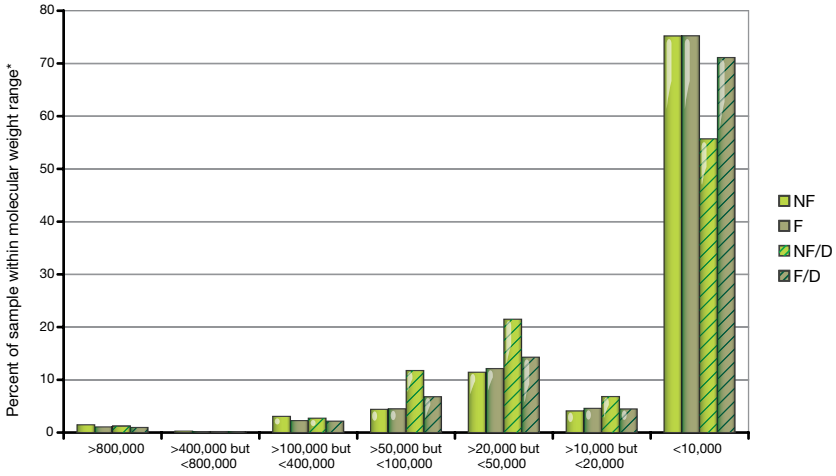
*Sugar percentages represent the average of duplicate or triplicate samples

Figure 3b.
Percent (w/w) sugar content of commercial aloe samples



*Sugar percentages represent the average of duplicate or triplicate samples

Figure 4a.
Molecular weight distributions of laboratory aloe samples



*Molecular weight percentages represent the average of triplicate samples

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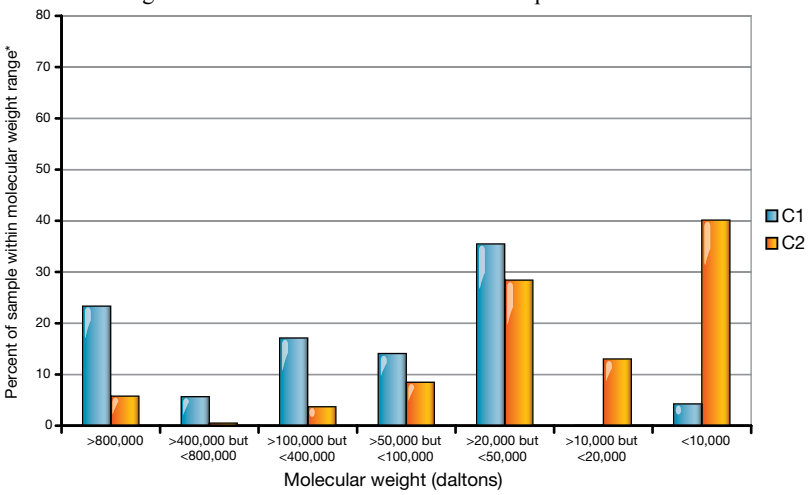
Figure 4b.
Molecular weight distributions of commercial aloe samples



*Molecular weight percentages represent the average of triplicate samples

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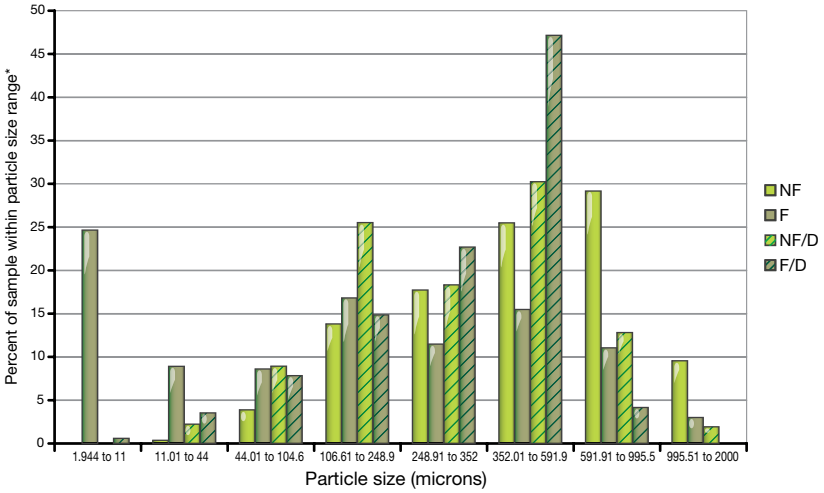
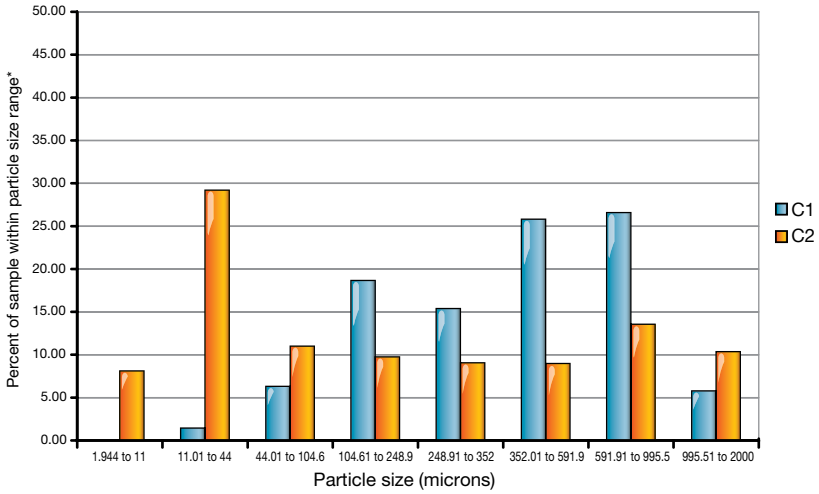
Figure 5a.
Particle size distributions of laboratory aloe samples



*Particle size percentages represent the average of triplicate samples

Figure 5b.
Particle size distribution of commercial aloe samples



*Particle size percentages represent the average of triplicate samples

A recent study reported that galacturonic acid is the predominant sugar in AVG cell walls.[2] The high percentage of glucuronic acid in G (24%) and C1 (16.5%) was an unexpected finding. The disappearance of glucuronic acid following laboratory blending suggests that mechanical disruption of cell walls releases enzymes (or ATP) which then convert glucuronic acid to glucose. The alcohol precipitation step in C1 commercial processing apparently preserves glucuronic acid, which is lost during C2 processing.

C1 commercial processing may be superior to C2 because it produces a higher MW powder of a larger PS enriched in acetylated mannans. Polysaccharide products with higher MW[3] or larger PS[4] may have greater effects on the immune system. Human oral ingestion of acetylated mannans have improved immune function in HIV-positive patients.[5,6]

CONCLUSIONS

AVG processing significantly impacts parameters believed to contribute to its biological effects: CC, MW and PS distributions. AVG processing must be considered when designing or interpreting studies investigating the impact of human oral ingestion of AVG supplements.

ACKNOWLEDGEMENT

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ABBREVIATIONS KEY

AV	aloe vera
AVG	aloe vera gel
C1	commercial preparation #1 (alcohol precipitation)
C2	commercial preparation #2 (blend of dehydrated and enzyme-treated AVG)
CC	carbohydrate composition
F	blended, filtered native gel
F/D	blended, filtered, freeze dried native gel
G	native gel
MW	molecular weight
NF	blended, not filtered native gel
NF/D	blended, not filtered, freeze dried native gel
PS	particle size

REFERENCE LIST

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