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Monitoring Physical and Chemical Properties of Freshly Harvested Field-grown *Aloe vera* Leaves. A Preliminary Report

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The content of total solids was obtained using the homogenate of mesophyll tissue of field-grown *Aloe vera* leaves. Filtered gel was used to determine the acidity (pH), content of total reducing sugars and amount of filterable solids. Additional cellulase-digested and filtered gel was used to determine optical density (for estimating aloin level) and concentrations of calcium and magnesium, as well as for HPLC analysis. There was a considerable variation in leaf size among different production fields. Acidity of the sap was rather consistent, ranging between 4.45 and 4.50. Fibre content, although small, varied by as much as 75%. Profiles of HPLC analyses showed two relatively stable and consistent peaks in nearly all samples that may be used to verify the presence of *Aloe* extract in an end product, provided these compounds are stable during processing of the *Aloe* gel.

Keywords: Aloe gel; sugars; aloin; HPLC analysis.

INTRODUCTION

For many years, products of *Aloe vera* (same as *Aloe barbadensis* Miller), whether these are the fresh gel, juice, or formulated products, have been used for health, medical and cosmetic purposes (Rowe *et al.*, 1941; Blitz *et al.*, 1963; Lee *et al.*, 1980; Norton, 1961). Some people keep one or a few plants at home to provide a readily available gel source for treatment of burns or other wounds. Others purchase cream, ointment, juice, etc. to meet their needs.

There are numerous products in the market, each claiming to contain *Aloe*. Some indeed have the genuine *Aloe* as claimed, whereas others may not contain *Aloe* or have lower amounts of *Aloe* than that indicated on their labels. Therefore, there is an urgent need for the *Aloe* industry to develop procedures and a reliable database, so that a product claiming to have *Aloe* can be tested and certified. This certification not only could reduce fraudulent claims, but also can build consumer confidence in *Aloe* products.

One problem in certifying various products is the lack of reliable data. The International Aloe Science Council (IASC), the certifying body of the *Aloe* industry, needs to know the content and levels of certain constituents in the raw materials and how these levels fluctuate with different harvest times. A study was initiated in April 1991 with the support from the IASC to develop certain baseline information which may be used by IASC to certify *Aloe* products.

MATERIALS AND METHODS

Starting 9 April, 1991, on each day, Monday through Thursday, one assigned grower of the four participating *Aloe vera* producers delivered three leaves collected from plants in a preselected $8.3 \times 8.3\ m^2$ field plot. Leaves were cut from plants early in the morning and sent to our laboratory usually arriving within 2 h. Leaf dimensions, including width and length, and weight were determined for each individual leaf. After washing, the leaves were blotted dry, and filleted to retain only the mesophyll tissue. Most leaves were processed on the same day they were delivered. However, occasionally they were refrigerated for 1–14 days before processing because of late delivery or other technical difficulties.

Leaf fillets were homogenized for 2 min in a household blender. Ten grams of the homogenate (including the pulp) was placed in a pre-weighed, dry glass petri dish, dried at 105 °C for 24 h and its weight determined for calculating the percentage of total solids. This crude gel was also used for the determination of pH.

The homogenate was filtered through a 2.0 μm cloth followed by Whatman No. 4 filter paper under vacuum. Ten grams of the filtrate was placed in a dry glass petri dish and dried at 105 °C and its dry weight determined. The difference between the dry weight of the crude gel and that of the filtered gel was defined as the fibre content.

The amount of total reducing sugars in the filtrate was determined by the use of copper sulphate and sodium potassium tartrate. A mixture of 50 mL of the filtered *Aloe* gel, 25 mL of the alkaline tartrate solution, and 25 mL of the copper sulphate solution was boiled and filtered. The precipitate (copper oxide) was collected, dried, and weighed. The amount of copper oxide was located in the Hammond Table to obtain the corresponding content of total reducing sugars (expressed in glucose equivalent) in the *Aloe* gel.

In another process, approximately 0.1 mg cellulase was added to the remaining crude gel and blended for 2 min at room temperature. The digested gel was filtered through Whatman No. 4 filter paper by gravi-

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Table 1. Characteristics of fresh *Aloe vera* leaves from four producers averaged over a 6-month period

Grower	Weight (g)	Leaf Length (cm)	Width (cm)	pH	Optical density (abs.)	Aloin (mg/L)	Soluble solids (%)[a]	Fibre (%)[a]	Sugars (mg/L)
1	478	53	9.7	4.47	1.095	31	0.708	0.077	2555
2	611	56	11.4	4.49	1.356	39	0.610	0.088	1441
3	704	60	11.5	4.51	1.020	29	0.675	0.088	2530
4	387	48	8.9	4.54	1.437	41	0.586	0.074	1361

[a] Percentage of fresh weight.

tation. The filtrate was filtered with a 0.5 μm prefilter and a 0.45 μm filter. 20 μL of the above filtrate was injected into a Waters HPLC (Millipore Corp., Milford, MA, USA) to obtain an analysis profile. The mobile phase consisted of 70% acetonitrile and 30% 0.05 M KH_2PO_4 at a flow rate of 1 mL per minute. The sample was chromotographed on an amino column (Cat. no. 8371, Alltech, Deerfield, IL, USA) and detected by absorption at 205 nm wavelength. The digested gel filtrate was also used to determine the optical density at 400 nm, from which the aloin content in the gel was determined by the use of a pre-established standard curve. In addition, a small amount of this refined gel was used for the determination of calcium and magnesium concentrations, which are considered to be related to the therapeutic effect of the *Aloe* gel, with an ICP Spectrophotometer.

RESULTS AND DISCUSSION

The average weights of individual leaves increased during the 33-week period (data not shown) and ranged between 390 and 700 g among growers (Table 1), possibly the result of different plant ages and culture practices. The acidity (pH) of the raw gel was fairly consistent (between 4.47 and 4.54). This was probably due to leaves being harvested in the early morning hours and the accumulated acids, as the result of CAM carbon fixation during the night, had not been consumed for the production of sugars (Bharucha and Joshi, 1957). Average aloin, which is considered a contaminant in *Aloe* juice, concentration was between 29 and 41 parts per million (ppm) and its level could have been reduced had the fillets been washed prior to blending. The average level of soluble solids was between 0.6% and 0.7% of fresh weight, where fibre ranged between 0.075% and 0.088% of fresh weight (Table 1). Average content of reducing sugars showed nearly a two-fold difference between the lowest (1441 mg/L or 0.14%) and the highest (2555 mg/L or 0.26%) concentrations.

The percentage of total soluble solids showed a general pattern of declining after week 13 (Fig. 1), although that of leaves from one producer fluctuated more widely than that of the other three. The concentration of reducing sugars showed a marked fluctuation with time, regardless of production site (Fig. 2). The small leaves from one producer had consistently lower levels of reducing sugars than the others. Total solids content (fibre and soluble solids) in the gel over a 33-

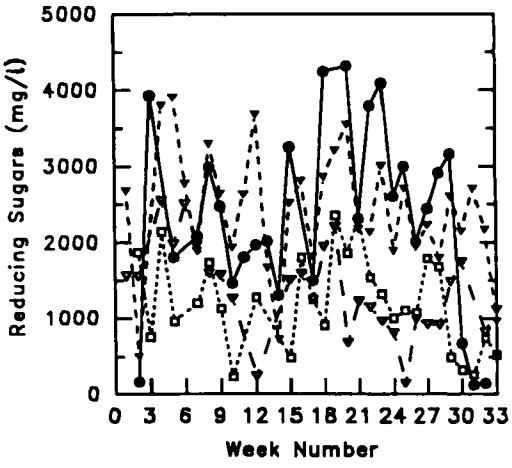

Figure 2. Concentration of reducing sugars (% fresh weight) in fresh *Aloe vera* gel from leaves of four producers.

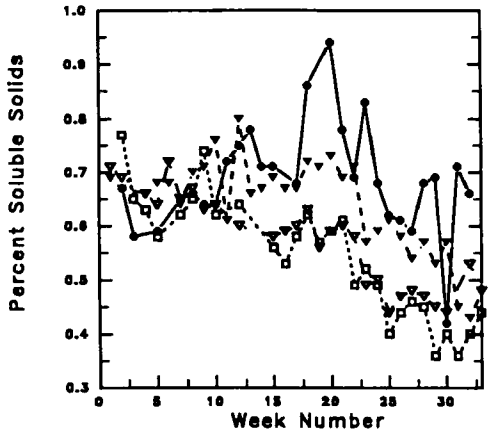

Figure 1. Content of soluble solids (% fresh weight) in fresh *Aloe vera* gel from leaves of four producers.

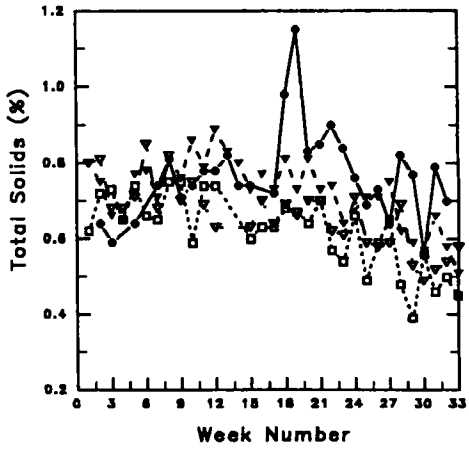

Figure 3. Total solids content (% fresh weight) in fresh *Aloe vera* gel from leaves of four producers.

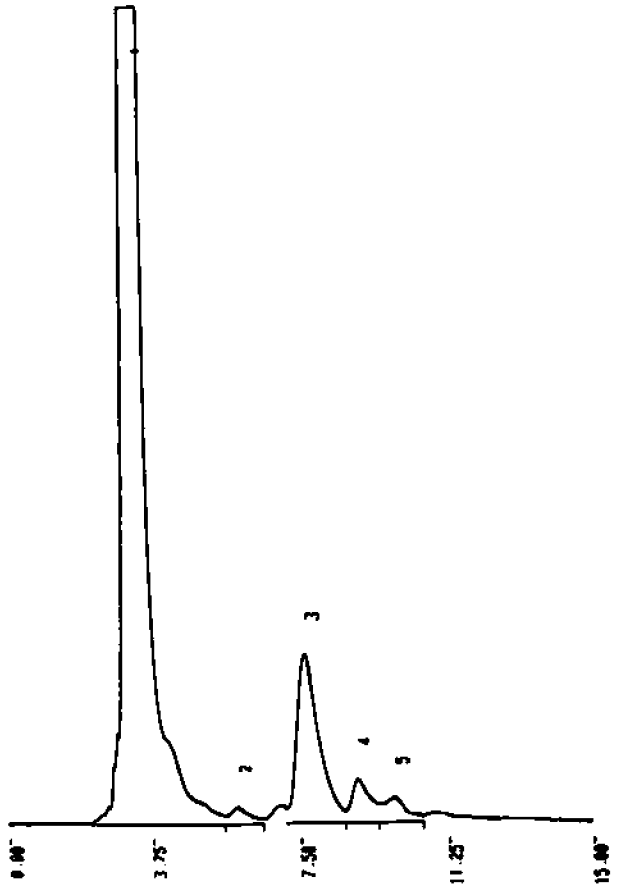

Figure 4. A typical HPLC output of a 20 µL of *Aloe vera* extract digested with cellulase. Peaks 3 and 5 are discussed in the text.

week period are presented in Fig. 3. The highs and lows followed closely with the profile of percentage of soluble solids. The low contents of solids and sugars in some weeks may have coincided with decreased sunlight during cloudy periods.

A common pattern of the HPLC profile is that samples all had several peaks near the front of the profile (short retention times), followed by a flat curve, and then the appearance of another group of lower peaks (Fig. 4). The interval between these two groups of peaks was about 3.7 min on our HPLC. The pattern and size of the front peaks differed markedly from one grower to another, but the downstream peaks were more stable. The size of the major front peaks declined

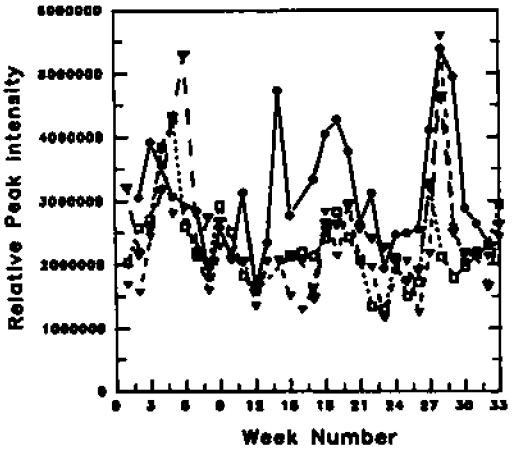

Figure 5. Relative concentration of a HPLC peak (no. 3 in Fig. 4) obtained from *Aloe vera* gel from leaves of four producers.

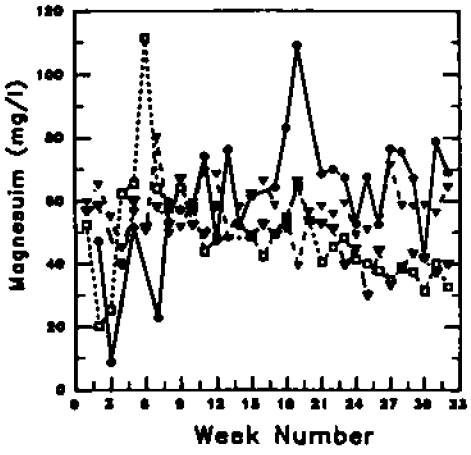

Figure 6. Magnesium concentration in *Aloe vera* gel extracted from leaves of four producers.

drastically starting week 23 (9 September, data not shown). Peak 3 (Figs 4 and 5) was present in all samples being processed and may be used as an indicator for the presence of *Aloe* in an end product. However, caution must be exercised since a compound corresponding to this peak is present in the extract of other species in *Liliaceae* such as the scales of *Lilium longiflorum* Thunb. (Easter lily) and the tuberous roots of *Asparagus densiflorus* (Kunth) Jessop (asparagus fern) processed similarly to *Aloe* leaves used in this study (Wang, unpublished data). Another peak (peak 5 in Fig. 4) is unique to *Aloe vera* and was not present in either Easter lily scale or asparagus fern root water extract. However, some *Aloe* samples processed between mid-September and mid-October did not display this peak (peak 5) in the second group of peaks. Using more than one peak to confirm the presence of *Aloe* gel in a product may be more reliable than using a single peak. There was no drastic change in the HPLC profile from samples that were tested on the day of harvest versus the profile of the extract being frozen for 30 days and thawed. At the present time, none of the HPLC peaks has been identified for *Aloe* gel.

The contents of calcium (Fig. 6) and magnesium (Fig. 7) both showed a significant variation among growers. Magnesium concentration remained relatively constant over time (between 30 and 70 mg/L), although it generally increased through the season in samples

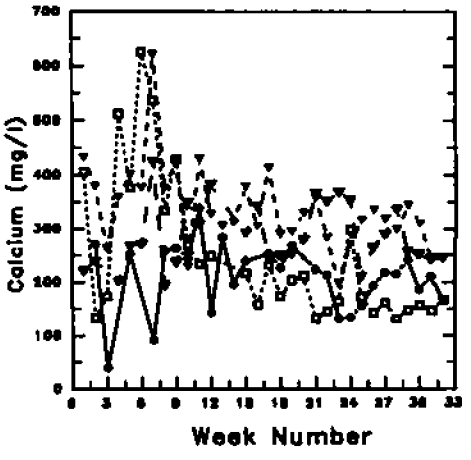

Figure 7. Calcium concentration in *Aloe vera* gel extracted from leaves of four producers.

from one producer. Calcium concentration declined slightly over the season. The fluctuation of these mineral concentrations did not clearly match with the time of rainfall or irrigation (data not shown).

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