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A Two-Year Study Monitoring Several Physical and Chemical Properties of Field-grown *Aloe barbadensis* Miller Leaves

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ABSTRACT

A study was initiated in April, 1991, to develop baseline information as part of a certification program for *Aloe barbadensis* products. Results generated constitute an initial data base for *Aloe barbadensis* fresh gel in order to develop attributes to be used in certifying product quality. The percentages of soluble solids and total solids and concentrations of Ca^{+2}, Mg^{+2}, Na^{+}, and K^{+}, as well as pH were measured over a two-year period. Seasonal fluctuations in some parameters were found in weekly sampling of fresh *Aloe barbadensis* leaves. Separation by HPLC of the freshly prepared *Aloe* extract or the frozen extract resulted in reproducible chromatographic profile with a unique peak.

RESUMEN

En abril de 1991 se inició un estudio para generar información básica como parte de un programa de certificación productos de *Aloe barbadensis*. Los resultados generados constituyen una base de datos inicial acerca del gel fresco de *Aloe barbadensis* para desarrollar atributos que puedan usarse en la certificación de la calidad del producto. Durante un período de dos años se evaluaron los porcentajes de sólidos solubles y sólidos totales, las concentractiones de Ca^{+2}, Mg^{+2}, Na^{+}, y K^{+}, así como el pH. Se encontraron fluctuaciones estacionales en algunos de los parámetros durante los muestreos semanales de hojas de *Aloe barbadensis*. La separación por medio de cromatografía líquida de alta presión (HPLC) del extracto fresco de *Aloe* o del extracto congelado dio como resultado un perfil cromatográfico reproducible con un pico único.

Aloe barbadensis Miller (also known as *Aloe vera*), a member of the family *Liliaceae*, is cultivated for its thick fleshy leaves from which many substances used in folk medicine and cosmetics are obtained. Historically, the yellow sap that exudes from the cut leaves has been used as a laxative or purgative (Cheney, 1970). *Aloe* gel has a reputation as a folk remedy for burns and wounds, and some people keep one or more plants available at home for this use. For more than fifty years, the gel in the parenchyma cells of the leaf has been processed and marketed as a drink product. Today, the gel is being used in many products such as fresh gel, juice, and other formulations for health, medical, and cosmetic purposes (Blitz *et al.*, 1963; Cera *et al.*, 1980; Genet and van Schooten, 1992; Morton, 1961).

Many *Aloe*-based products on the market, such as creams, ointments, juices, facial tissue, and shampoo, contain *Aloe* as claimed; whereas others may not contain or may have less *Aloe* than shown on their labels. The *Aloe* industry needs a way to police itself by developing test procedures and a reliable database so that products claiming to have *Aloe* can be confirmed by testing (Waller, 1992). This certification not only could reduce fraudulent claims, but also would build consumer confidence in *Aloe* products.

Of major importance in developing this database is knowledge of the gel contents and levels of certain constituents in the raw *Aloe* materials and how these levels fluctuate with time of year and locations. This study was initiated to develop certain specific baseline information that could be used for certifying *Aloe* products. A preliminary report covering the first 33 weeks of data was previously published (Wang and Strong, 1993).

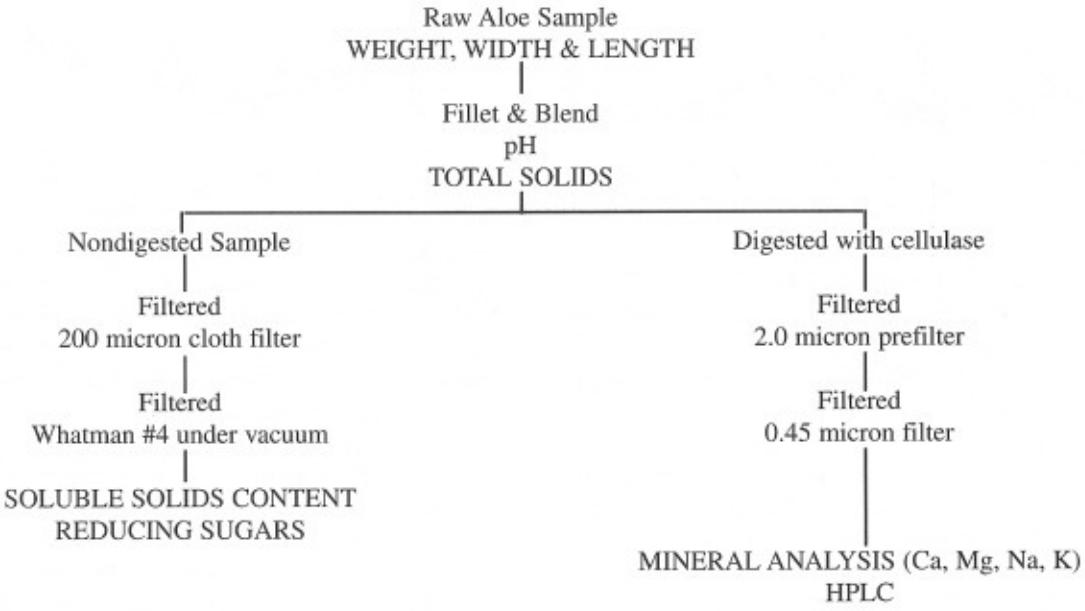
MATERIALS AND METHODS

Processing of specimen. This study began on 9 April 1991. On each Tuesday through Thursday, one assigned grower of three participating *Aloe barbadensis* producers delivered three leaves collected from plants in a preselected 8.3 x 8.3 m^2 field plot. The leaves were harvested early in the morning and arrived at our laboratory within two hours. The leaves were washed, and leaf width, length, and weight were measured for each individual leaf. Leaves were normally processed on the same day they were delivered but, on occasion the leaves were refrigerated at 4-5°C for 1-14 days before processing because of late delivery or other technical difficulties. The fresh *Aloe* leaf, when cut from the plant soon seals the wound by exuding yellow sap. Whether or not kept under refrigeration, the whole leaf will remain turgid for some time without significant weight loss or apparent deterioration.

The three leaves were filleted and the translucent mesophyll tissues from each leaf were combined into a single composite sample. The sample was homogenized for two minutes in a household blender and then the laboratory procedure given in Figure 1 was followed.

Total and soluble solids. The homogenate was used for the immediate determination of gel pH. A 10-gram sample of the homogenate (including the pulp) was placed in a dry, preweighed glass petri dish, dried at 105°C for 24 hours and

Figure 1. *Aloe barbadensis* laboratory procedures flow chart.



weighed to determined the percentage of total solids.

One half of the remaining homogenate was filtered through a 200 µm cloth followed by a Whatman No. 4 or an equivalent filter paper (20-25µm pore size) under vacuum. A 10-gram sample of this filtrate was placed in a dry, pre-weighed glass petri dish, dried at 105°C for 24 hours and weighed to calculate the percentage of soluble solids. The dried gel forms a thin layer of a yellowish-brown film firmly attached to the glass that feels dry to the touch. The difference between the dry weight of the crude gel and that of the filtered gel was defined as the fiber content.

Reducing sugars. The amount of total reducing sugars in the filtrate was determined by using copper sulphate (69.28 g•liter^{-1}) and alkaline tartrate solution (346 g•liter^{-1} $C_4H_4Na0_6$ •$4H_20$ and 100 g•liter^{-1} NaOH). Fifty ml of the filtered *Aloe* gel, 25 ml of the alkaline tartrate solution, and 25 ml of the copper sulphate solution were boiled for two minutes and then cooled. The solution was filtered and the precipitate (copper oxide) collected, dried and weighed. The weight of the copper oxide was located in the Hammond Table to obtain the corresponding content of total reducing sugars, expressed in glucose equivalent, in the *Aloe barbadensis* gel (AOAC, 1984).

The remaining half of the crude, nonfiltered sample was digested with approximately 0.1 mg cellulase and blended for two minutes at room temperature. The cellulase breaks the ß1-4 linkages between the simple sugar molecules and thereby eliminates the viscosity. The digested gel was filtered through a Whatman No. 4 filter paper by gravity. A sample of approximately 30 ml of the filtrate was filtered with a 2.0 µm prefilter and a 0.45 µm filter. A partial chromatographic separation of the extract was obtained by injecting 20 µl of the filtrate into a Waters HPLC model 510 (Millipore Corp., Milford, MA). The mobile phase consisted of 70% acetonitrile and 30% 0.05 M KH_2PO_4 at a flow rate of 1.0 ml•min^{-1}, at 32°C, and 10.4 Mpa pressure (1500 psi). The sample was chromatographed on a bonded amino acid column (Cat. No. 8371, Alltech, Deerfield, Ill) and the analyte detected by UV absorption at a wavelength of 205 nm (V^4, ISCO, Inc., Lincoln, Neb).

Minerals. Cellulase treated gel was used for the determination of the Ca^{+2} and Mg^{+2} concentrations with an Inductively Coupled Plasma Emission Spectrophotometer (ARL 3510, Applied Research Lab, Sonland, Calif.). Beginning with week 20 of the study, the Na^+ and K^+ concentrations of the gel were also determined.

RESULTS AND DISCUSSION

Figure 2. Average leaf weight of *Aloe barbadensis* over a period of 112 weeks.

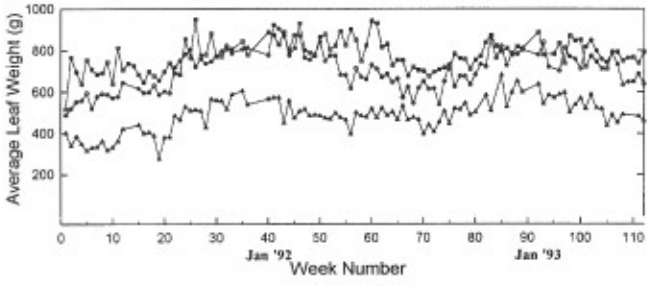


Figure 3. Weekly pH of *Aloe barbadensis* gel from 3 growing areas.

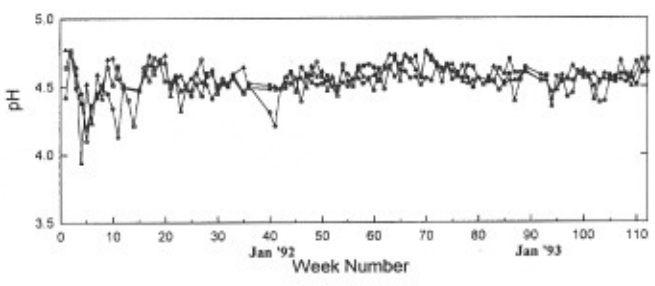


Figure 4. Average percentage soluble solids of hand filleted *Aloe barbadensis* gel.

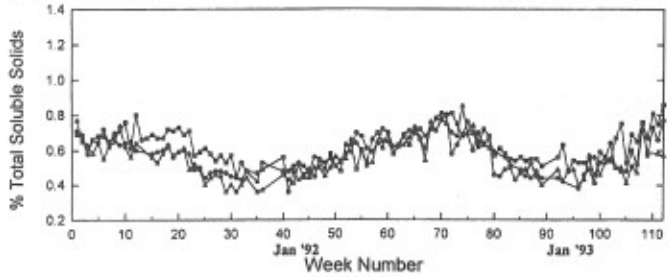
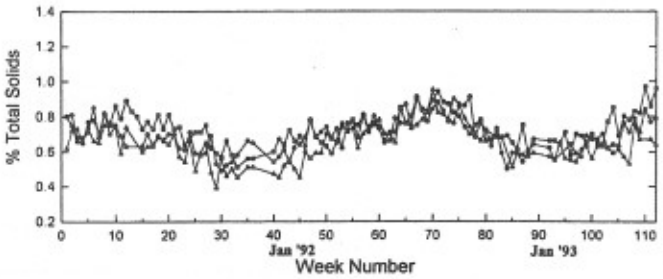


Figure 5. Average percentage total solids of hand filleted *Aloe barbadensis* gel.

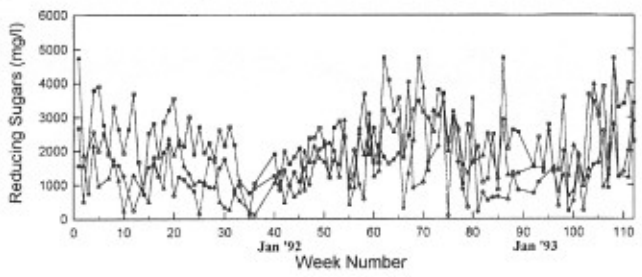


Weight. The average weights of individual leaves slightly increased the last three months of the year and slightly decreased during the summer months over the two-year period. Most *Aloe barbadensis* is grown without supplemental irrigation, lighter leaves in the summer months may be the result of water loss due to high temperatures and heat stress. The average leaf weight ranged between 485 and 774 g among growers (Table 1), possibly the result of different plant ages and cultural practices among locations and growers. The average weight of leaves from grower 3 was consistently less than the others possibly due to younger plants and different cultural techniques (Fig. 2).

Acidity. The range of gel acidity (pH) was between 4.4 and 4.7, averaging 4.55 (Table 1, Fig.3). The high acidity was likely due to the leaves being harvested in the early morning hours. In *Aloe barbadensis*, organic acids accumulate as the result of crassulacean acid metabolism (CAM) carbon fixation during the night. The acids are then used for the production of sugars (Bharucha and Joshi, 1957). The fluctuation of pH in the translucent leaf tissue during the day was not measured.

Total and soluble solids. The two-year average soluble solids content for the study samples was 0.58% ± 0.11% (grand mean±SD) of fresh weight. The average from farm to farm ranged from 0.55% to 0.62%. Fiber content ranged between 0.09% and 0.12% of fresh weight from farm to farm (data not shown). The percentage of soluble solids showed a general pattern of reduction in the winter months and increase in the summer months (Fig. 4). This pattern is also seen in the percentage total solids (averaged at 0.69%±0.10% for the two-year study samples) that includes soluble solids and fiber (Fig. 5). The lower contents (averaged near 0.5% in January) of total solids and sugars in the winter may have coincided with decreased day length and decreased sunlight during cloudy periods causing increased water retention, reduced water loss, or lower net photosynthesis. Light energy caught during the daylight hours is needed to fix carbon at night in plants having CAM photosynthesis. Higher solids contents in summer (near 0.8% in July) may be the result of greater water loss that was not replenished by water uptake due to the heat and dry soil. Amounts of rainfall and irrigation were not accurately recorded at the various sampling plots. As a consequence, a correlation between rainfall or irrigation and solids content could not be established.

Figure 6. Seasonal and weekly variation in *Aloe barbadensis* gel reducing sugars.



Reducing sugars. Reducing sugars ranged from 1466 (0.15%) to 2391 mg•liter^{-1} (0.24%) from farm to farm over the two-year period. The concentration of reducing sugars showed a marked fluctuation with season, regardless of production site, and declined during the winter months of 1991 (Fig. 6). Consequently, the concentration of total reducing sugars may not be a reliable indicator for determining the amount of *Aloe* in a product.

HPLC analysis. A common pattern among the HPLC profiles was that all *Aloe barbadensis* leaf extracts had several sharp peaks at the solvent front (short retention times of less-absorbed molecules) followed by a relatively flat portion, and then the appearance of one large downstream peak surrounded by a few smaller ones (Fig. 7). As the chromatograph column ages, it loses its absorption capacity, and the interval between these two groups of peaks shortens. The pattern and size of the front peaks differed markedly from one grower to another and varied over time. However, the downstream peaks were more similar.

Peak 5 (or peak "E" when named in an alphabetical order; Figs. 7 and 8) was present in all samples being processed and might be used as an indicator for the presence of *Aloe barbadensis* in some products. This peak is not found in processed *Aloe barbadensis* gel that has been bacterially contaminated or in leaf extract that has been stored for an extended period of time without refrigeration. However, caution must be exercised in using this peak as an identifier for *Aloe*

Figure 7. A typical HPLC output of *Aloe barbadensis* gel extract digested with cellulase.

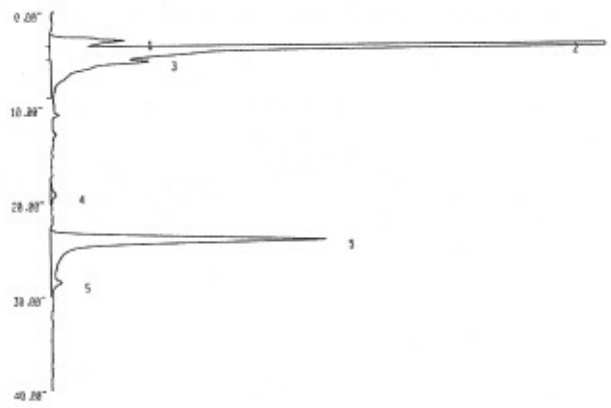
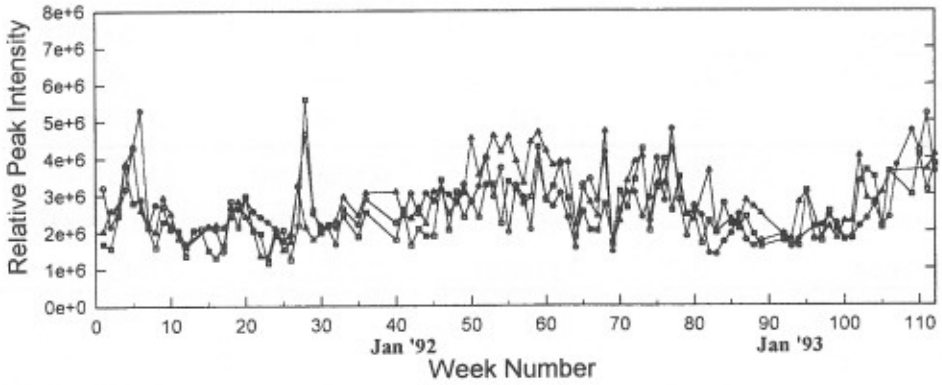
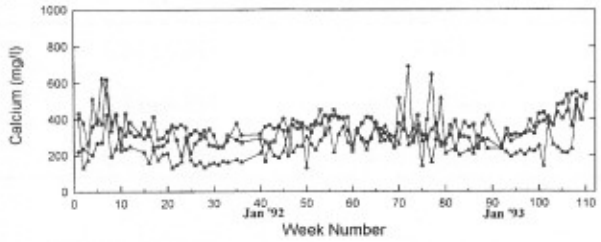


Figure 8. Relative concentration of a HPLC peak (no.5 in Fig.7) obtained from *Aloe barbadensis* gel.



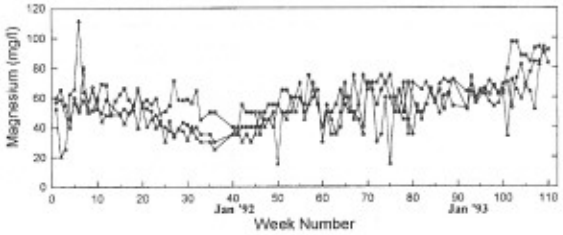
since a compound corresponding to this peak is present in the extract of other species in *Liliaceae* such as the scales of *Lilium longiflorum* Thunb. (Easter lily) and the tuberous roots of *Asparagus densiflorus* (Kunth) Jessop (asparagus fern) processed similarly to *Aloe* leaves used in this study (Wang, unpublished data). In addition, injecting 20µl of 1100 mg•liter-1 D(+) malic acid (Sigma Chemical Co., St.Louis, MO) produces a profile that shared a large downstream peak having the same retention time with the fresh extract of *Aloe*. Leaves of *Sedum* and *Phalaenopsis* orchid (plants having CAM carbon metabolism), as well as green apple are known to contain high concentrations of malic acid. Injections of their juices into the HPLC produced a large downstream peak at the same retention time as *Aloe barbadensis* gel. A small peak (peak 6) following peak 5 was present in the HPLC profiles in nearly all extracts tested. Therefore, adjusting recorder attenuation and using multiple peaks to confirm the presence of *Aloe* gel in a product should be more reliable than using solely peak 5. Although the biomedical effectiveness might decline with time, there was no drastic change in the HPLC chromatographic separation of the extract from samples tested on the day of harvest, stored as whole leaves under refrigeration for two weeks, or prepared extract being frozen for up to two years and thawed.

Figure 9. Calcium concentration in *Aloe barbadensis* gel extracted from leaves of 3 producers.



Metal cations. The contents of Ca^{+2} (Fig. 9) and Mg^{+2} (Fig. 10) showed a significant variation among growers. Ca^{+2} concentration ranged from a high of 690 mg•liter^{-1} and a low of 130 mg•liter^{-1}. One grower who reported using irrigation had consistently lower calcium levels than the others during certain periods. Mg^{+2} concentration averaged 55 mg•liter^{-1} . A slight decline in Mg^{+2} was noted in the fall of 1992 for two of the growers. Once calcium enters a plants tissue, in contrast to magnesium, it is not remoblized out of that tissue. Excessively high concentrations of Ca^{+2} in the soil can cause a decline in the uptake of Mg^{+2} by plant roots. But this antagonism was not detected between the Ca^{+2} and Mg^{+2} concentrations. Na^{+} (Fig. 11) and K^{+} (Fig. 12) concentrations also showed variation among growers and over time. Na^{+} concentration averaged 187 mg•liter^{-1} but ranged from a high of 462 to a low of 80 mg•liter^{-1}. K^{+} concentration averaged 378 mg•liter^{-1}, ranging from 690 to 115 mg•liter^{-1}. The fluctuation of these mineral concentrations did not clearly match with the time of rainfall or irrigation practice (data not shown).

Figure 10. Magnesium concentration in *Aloe barbadensis* gel extracted from leaves of 3 producers.



Summary. The parameters found in two-year weekly study of samples of fresh *Aloe barbadensis* leaves show seasonal and grower to grower fluctuations. Over the 112-week study period, the HPLC chromatograph indicated two downstream peaks common in freshly prepared or fresh frozen *Aloe*

Figure 11. Sodium concentration in *Aloe barbadensis* gel extracted from leaves of 3 producers.

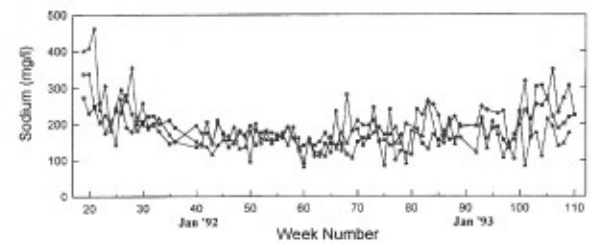


Figure 12. Potassium concentration in *Aloe barbadensis* gel extracted from the leaves of 3 producers.

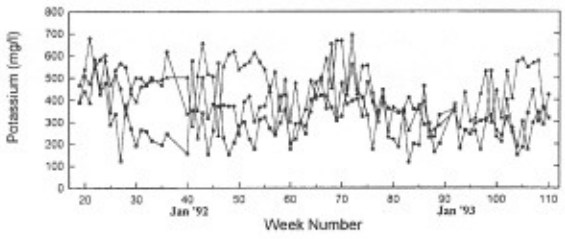


Table 1. Characteristics of fresh *Aloe barbandensis* leaves from three producers averaged over a 112 week period.

	Leaf				Soluble	Total	
	Weight	Length	Width		solids	solids	Sugars
Grower	(g)	(cm)	(cm)	pH	(%)[z]	(%)[z]	(mg•liter^{-1})
1	705±9.5[y]	59.5±0.60	12.2±0.45	4.53±0.01	0.575±0.010	0.691±0.009	1466±87.5
2	774±7.3	60.7±0.30	12.1±0.06	4.56±0.01	0.617±0.010	0.727±0.009	2391±96.7
3	465±7.8	55.5±0.54	9.5±0.07	4.57±0.01	0.555±0.010	0.646±0.011	1713±97.7

[z]Percentage of fresh weight.

[y]Mean±S.E.

gel. The information generated in this research should facilitate the development of product standards in the *Aloe* industry. However, when using these data, other than taking the long-term average as a standard for comparison, one should not ignore the variation among samples and producers and the range of each parameter in question.

ACKNOWLEDGEMENT

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Table 2. Mineral concentrations of fresh *Aloe barbadensis* gel from three producers average over a 112-week period.

	Mineral concentration (mg•liter^{-1})			
Grower	Ca	Mg	Na	K
1	330.6±9.7[z]	51.8±1.5	194.8±6.8	370.1±14.4
2	347.7±7.2	60.1±1.3	182.3±4.1	313.4±11.0
3	260.3±9.5	54.0±1.6	180.8±7.4	449.5±12.0

[z]Mean±S.E.

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