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Anion Composition of Açaí Extracts

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Supporting Information

ABSTRACT: Many products labeled açaí are presently marketed as natural supplements with various claimed health benefits. Authentic açaí is expensive; as a result, numerous products labeled as containing açaí are being sold that actually contain little or no açaí. Authentic açaí samples from Brazil and Florida as well as several reputed açaí products were analyzed by suppressed conductometric anion chromatography. Columns with different selectivities were used to obtain a complete separation of all anions. Tandem mass spectrometry was used for confirmation of the less common ions. Quinate, lactate, acetate, formate, galacturonate, chloride, sulfate, malate, oxalate, phosphate, citrate, isocitrate, and *myo*-inositol hexakisphosphate (phytate) were found. Only the Florida açaí had detectable levels of hexanoate. No açaí sample had any detectable levels of tartrate, which is present in abundance in grape juice, the most common adulterant. The highly characteristic anion profile and in particular the absence of tartrate can readily be used to identify authentic açaí products. Açaí from Florida had a 6 times greater level of phytate. The present analytical approach for phytate may be superior to extant methods.

KEYWORDS: *ion chromatography, açaí, malate, tartrate, phytic acid, inositol hexaphosphate*

INTRODUCTION

Açaí palms (*Euterpe oleraceae* Mart.) are native to Central and South America and grow from Belize to Peru and Brazil.[1] Most of the genuine commercial açaí fruits come from the Brazilian Amazon rainforest; commercial cultivation has also begun in Florida. The berries range from green to dark purple in color, and ~90% of the mass of the berry is the pit. The health and wellness benefit claims surrounding açaí straddle the border of fact and fantasy; cure-all claims are all too common. There is, however, credible evidence that it has very high antioxidant content[2,3] and can remove reactive oxygen species,[4] inhibit nitric oxide (NO) synthase[5] and cyclooxygenase (COX)-1 and -2,[6] protect human vascular endothelial cells against oxidative stress and inflammation,[7] and induce apoptosis in HL-60 leukemia cells in vitro.[8] Indeed, the name açaí sells many products.

Despite many "açaí juice" products being on the market, strictly speaking, no "juice" can really be produced from the berry, if juice is defined as the aqueous product of squeezing the berry. Açaí is similar to olives in being oleaginous; oil, not juice, is produced on pressing. Instead, the outer portion of the fruit is scraped off the relatively large seed; water is then added to the berries to produce a purée that is often sold frozen. Depending on the amount of water added, açaí grosso (thick), médio (middle), or fino (fine) grades are produced, with the following respective amounts of total solids: >14, 10−14, and <10%.[9] Authentic açaí products or supplements are expensive. They are also often sold in the freeze-dried form. Given the cost of the genuine product, the incentive for adulteration is high, and given the perceived benefits, the market is significant. For other high-value products with perceived health benefits, such as pomegranate juice, international authenticity specifications have been set.[10] There are many cautionary notes on the Web[11] and elsewhere regarding the authenticity of açaí products, and açaí berry scams have been the subjects of lawsuits by several state attorneys general in the United States as well as the U.S. Federal Trade Commission.[12]

At this time few data are available for the characteristic composition of açaí. Relative to other analysis techniques, suppressed conductometric anion chromatography (hereinafter called SCAC) provides rapid and sensitive analysis. Whereas reversed phase (RP) or ion-pair (IP) liquid chromatography may determine both ionic and nonionic species, this will make for a more complex chromatogram. On the other hand, with SCAC, the number of anionic analytes (the corresponding acids must have a pK_a~ <6 to be detected) is obviously more limited. Also, RP or IP chromatography requires an expensive mass spectrometer as a detector; many of the analytes of interest (e.g., organic acids without unsaturated linkages) absorb poorly in the UV to be sensitively detected by optical absorption. In contrast, virtually all carboxylic acids ionize sufficiently in SCAC to be detected. In SCAC, the retention behavior of most of the anions is known for a number of different commercially available ion exchange columns, at least under some particular elution conditions, and can be predicted under most others.[13] If there are ions not identifiable on the basis of known retention

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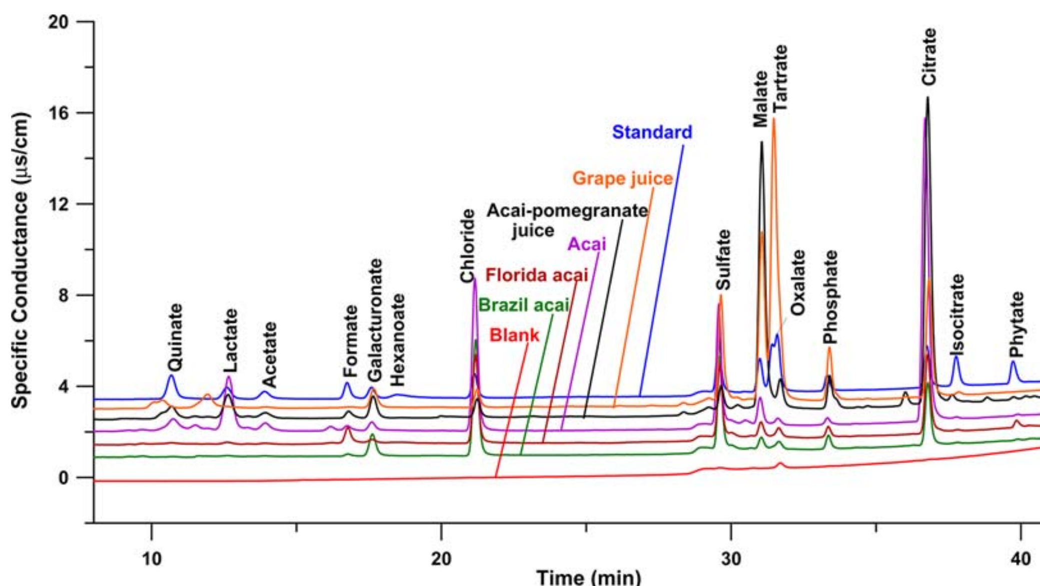

Figure 1. Chromatograms of blank, samples, and standards on the AG24-AS24 column set. Blank, DI water; standard mixture, 20 μM quinate, 10 μM lactate, 10 μM acetate, 10 μM formate, 27 μM hexanoic acid, 10 μM galacturonate, 10 μM chloride, 10 μM sulfate, 10 μM malate, 10 μM tartrate, 10 μM oxalate, 10 μM phosphate, 10 μM citrate, 10 μM isocitrate, 2 μM phytate.

behavior, they can be identified by tandem mass spectrometry (MS/MS). Once they are identified, further use of a mass spectrometer is not essential.

The ionic composition of açaí in general and its organic acid profile in particular has never previously been described. General approaches to the characterization of organic acid content of fruit (primarily grape) juices were reviewed some time ago.[14] That review tabulated conditions of liquid chromatographic (HPLC) and electrophoretic methods. Several gas chromatography (GC) methods were also described earlier.[15,16] Attractive techniques were developed to trap the analytes on anion exchange resins, methylate them in situ, and perform supercritical fluid extraction prior to GC analysis.[17] The general availability of GC-MS in many laboratories makes this particularly attractive; direct analysis without derivatization is possible in many cases.[18] Infrared spectroscopy, in both mid-IR[19,20] and near-IR,[19] has proven useful in determining major organic acids (and often other constituents) in a production setting.

These approaches are not without problems; many acids are thermally labile at GC temperatures, and IR spectrometry is too insensitive to determine the acids present in lower levels. Liquid phase separations, especially HPLC, have therefore been primarily used for fingerprinting.[21] Capillary electrophoresis (CE) has been used for this analysis with[22] or without[23,24] prior derivatization, but the use of HPLC has dominated this application.[25−31] Although some approaches use prederivatization prior to a RP separation,[32] most do not.

Most reported approaches use RP columns with a low pH eluent to keep the analyte acids significantly in the un-ionized form; the basic separation mechanism is ion exclusion,[33,34] with stronger acids eluting first. Some approaches directly use strong acid form cation exchangers and a mineral acid eluent to accomplish the same ends.[35−37] Ion pairing agents such as tetrabutylammonium are applicable[26] but have not been commonly used. UV absorbance measurement singly[38] or simultaneously/sequentially at multiple wavelengths has been used,[33,39−45] but suppressed conductivity and mass spectrometry provide much better limits of detection (LODs).

We aim in this work to characterize the anion profile of both Brazilian and Florida açaí and thence to determine if this can be used for authentication.

■ MATERIALS AND METHODS

Brazilian açaí berries were bought directly in a market in Belém, Pará, Brazil. They were washed and disinfected with chlorinated water and then transferred to a pulping apparatus to obtain the edible portion (pulp + peel). Water was added to obtain "açaí médio", followed by freezing. The frozen pulp (including added water) was lyophilized in the author's laboratory in Rio de Janeiro to obtain a powder. Lyophilized açaí powder was also obtained from an açaí cultivation farm in Florida. Both samples were sent to the Kansas City district laboratories of the U.S. Food and Drug Administration (FDA). Various other characterizations of these samples (e.g., lipid composition), not discussed here, were carried out at the FDA. Ion chromatographic analysis was conducted at the University of Texas at Arlington. In addition, a sample of white grape juice (brand W), a beverage sample labeled açaí (brand S, did not claim to be a juice and contained undissolved settled solids, only the decantate was used for analysis), and another labeled açaí−pomegranate juice (brand O) were bought from local grocery stores in Arlington, TX. Both the açaí and açaí−pomegranate juices were dark purple in color.

Accurately weighed samples (∼28 mg) of the lyophilized açaí powders were transferred to a 25 mL volumetric flask, Milli-Q deionized water was added to the mark, and the contents were sonicated for 20 min. The liquid was divided into two equal portions and was then centrifuged at 7650 rpm for 20 min, followed by filtration through a 0.2 μm nylon syringe filter (13 mm). The filtered sample was diluted 4-fold before IC analysis.

The store-bought "juice" samples were filtered through a Hi-Load C-18 Sep-Pak column (www.waters.com) and then a 0.2 μm syringe filter. The açaí−pomegranate juice and grape juice were diluted 200-fold, whereas the açaí extract was diluted 100-fold prior to IC analysis.

Chromatography was conducted on an ICS2000 ion chromatograph, equipped with an ASRS Ultra II 2 mm anion self-regenerating SRS Suppressor and a CD 25 conductivity detector with a DS3-1 detector cell. Three different sets of guard and separator columns were examined for the desired separation: AG11+AS11, AG24+AS24, and AG15+AS15, all 2 mm in diameter. All of the above were from www.dionex.com. The sample injection volume was 20 μL. Gradients used on each column were optimized for the best separation; the KOH

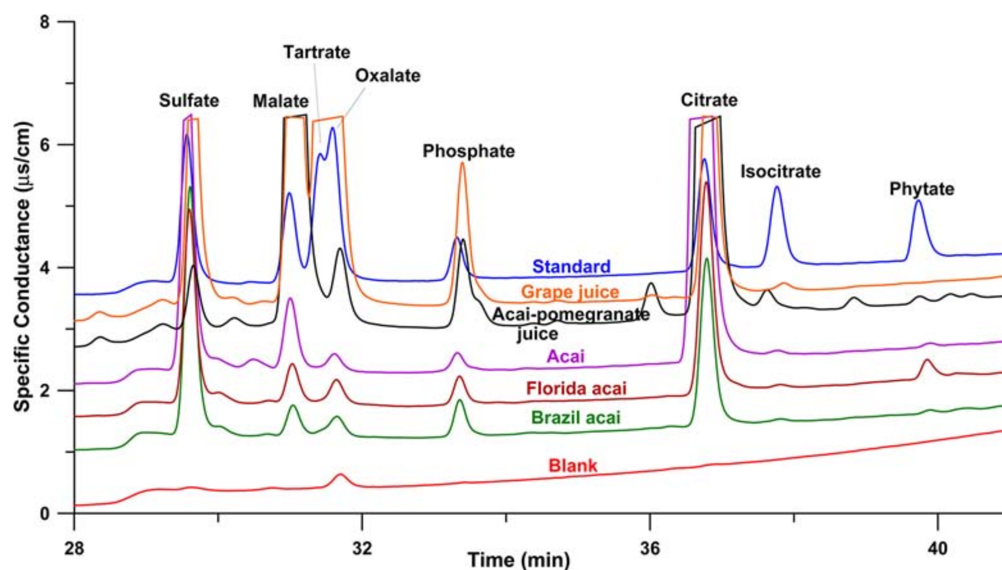

Figure 2. Detailed view of the 28–41 min region of Figure 1.

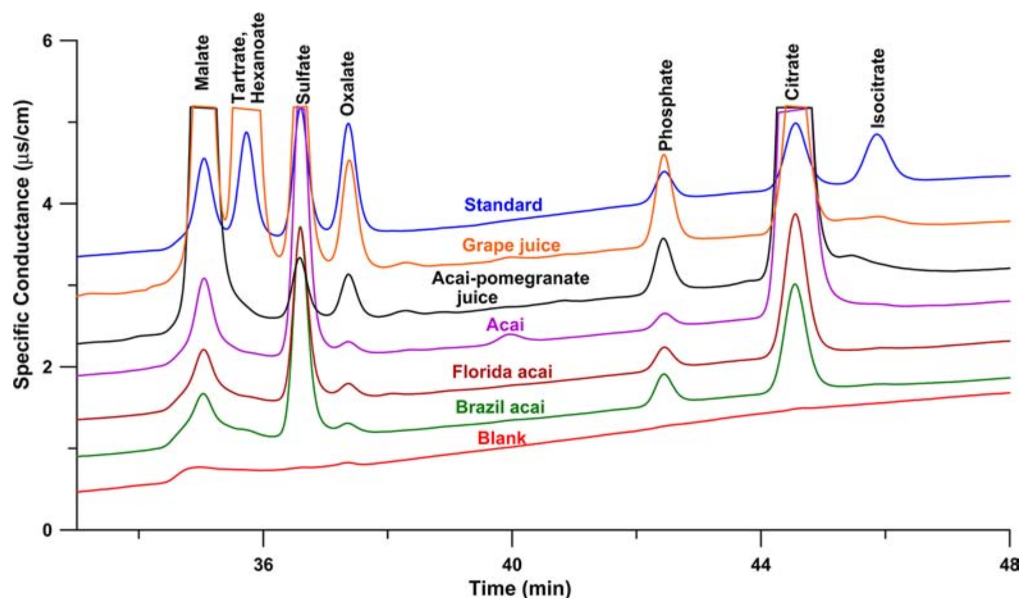

Figure 3. Chromatograms of blank, samples, and standard mixture (see Figure 1) in the 33–48 min region on the AG15-AS15 column set.

gradient elution programs used with each column set is presented in Table S1 in the Supporting Information. The background conductivity was usually from 0.8 to <3 μS/cm for gradients of 2.5–80 mM KOH.

IC-MS/MS was performed using the AG24-AS24 column sets and a TSQ Quantum Discovery Max triple-quadrupole mass spectrometer equipped with enhanced mass resolution and heated electrospray ionization probes. A model AS autosampler (all MS equipment from www.thermo.com) was used for convenience. The injection volume was 2 μL for selected ion monitoring (SIM) mode, whereas 25 μL was used to obtain high-resolution and fragmentation spectra. Instrument parameters were as follows: electrospray voltage, 3 kV, vaporizer temperature, 350 °C; capillary temperature, 250 °C; skimmer offset, 0 V; collision gas pressure (fragmentation only), 1.5 mTorr; collision energy, 10 V; and chromatographic filter, 45 s. Thomson (Th) units are used for m/z hereinafter. For fragmentation of hexanoic acid in the scan-centroid mode, the parent ion was at 115 Th, and the scan range was 30–114 Th over 0.200 s. For fragmentation of phytic acid in the scan-centroid mode, the parent ion was at 218.96 Th, and the scan range for segment 1 was 30–218 Th over 0.200 s and that for segment 2 was 221–660 Th over 0.300 s. In both above cases Q1 and Q3 resolutions were 0.7 and 0.3 Th, respectively, in full width at half-maximum (fwhm). The SIM/profiling mode utilized Q1 and Q3 resolutions of 0.5 and 0.4 Th fwhm, respectively. The high-resolution scan for the phytic acid in profile mode used Q1 resolution set at "pass" and Q3 resolution at 0.04 fwhm. The scan range was 218.5–220 Th over 0.100 s.

RESULTS AND DISCUSSION

Chromatographic Separation. Standards containing quinate, lactate, acetate, formate, hexanoate, galacturonate, chloride, sulfate, malate, phytate (*myo*-inositol hexakisphosphate), oxalate, tartrate, phosphate, citrate, and isocitrate (hereinafter called the standard mixture), as well as samples of interest, were analyzed on all three column sets. The resulting chromatogram on the AS24 column is shown in Figure 1, and the 28–40 min region of this chromatogram is shown in magnified view in Figure 2. Phytate is the last peak to elute at ~40 min. Figure S1 in the Supporting Information and Figure 3 show the corresponding chromatograms for the AS15

Table 1. Concentrations (Milligrams per Gram) of Anions in Açaí[a]

analyte ion	Florida açaí	Brazilian açaí	açaí	açaí−pomegranate juice	grape juice
quinate	0.555 ± 0.012	0.192 ± 0.034	0.185 ± 0.006	0.366 ± 0.013	<0.003
lactate	0.413 ± 0.022	0.202 ± 0.015	0.308 ± 0.006	0.251 ± 0.005	<0.001
acetate	0.260 ± 0.012	0.136 ± 0.003	0.046 ± 0.002	0.067 ± 0.005	0.014 ± 0.002
formate	1.58 ± 0.01	0.228 ± 0.014	0.012 ± 0.001	0.038 ± 0.003	0.007 ± 0.001
galacturonate	1.83 ± 0.14	12.3 ± 0.2	0.126 ± 0.001	0.661 ± 0.026	0.565 ± 0.014
hexanoate	1.55 ± 0.12	<0.108	<0.003	<0.006	<0.006
chloride	4.09 ± 0.01	5.52 ± 0.04	0.200 ± 0.000	0.048 ± 0.002	0.047 ± 0.001
sulfate	4.18 ± 0.07	5.44 ± 0.12	0.190 ± 0.001	0.067 ± 0.001	0.322 ± 0.002
malate	2.01 ± 0.08	1.81 ± 0.13	0.103 ± 0.004	2.52 ± 0.03	1.44 ± 0.02
tartrate	<0.016	<0.016	<0.001	<0.001	1.92 ± 0.05
oxalate	0.38 ± 0.02	0.214 ± 0.020	0.013 ± 0.001	0.072 ± 0.002	0.184 ± 0.006
phosphate	1.92 ± 0.05	2.75 ± 0.72	0.042 ± 0.001	0.406 ± 0.007	0.576 ± 0.007
citrate	15.7 ± 0.1	13.1 ± 0.8	1.90 ± 0.03	3.67 ± 0.02	1.31 ± 0.02
isocitrate	0.255 ± 0.016	0.278 ± 0.008	0.009 ± 0.0001	<0.002	0.036 ± 0.002
phytate	1.34 ± 0.01	0.229 ± 0.010	0.007 ± 0.0003	0.025 ± 0.001	<0.003

[a]All results listed as average ± standard deviation (n = 3). The ions are listed in order of elution.

column set. In this case, phytate does not elute within an hour. Dissolved CO_2 appears as the carbonate peak in all samples; it is a broad peak and can obscure the elution of some peaks of interest. This can be seen in Figure S2 in the Supporting Information for the malate peak on the AS15 column. Blank, standard mix, and sample chromatograms on the AS11 column are shown in Figure S3 in the Supporting Information. Carbonate and tartrate coelute under these conditions (we also could not separate them on this column under all other conditions we tested), and this peak in turn elutes very close to malate. The separation from malate shown here was the best separation observed.

Although a relatively low eluent concentration (2.5 mM KOH) was used for all three columns for the first 10 min, the early-eluting ions lactate, acetate, and formate were well separated only on the AS24 column (Supporting Information, Figure S4). However, tartrate and oxalate elute too close to each other on this column under this condition; accurate quantitation will be impossible if both are present. One critical question (see below), if tartrate is present at all, can likely be answered nevertheless, especially if a standard containing both is run at the same time (compare the traces for the standard mix and the açaí sample traces in Figure 2). However, tartrate was well separated from all other anions (except for hexanoate) only on the AS15 column. Hexanoate was observed in only the Florida açaí and that in trace amounts. Accurate determination of tartrate should thus be possible by the stated method on the AS15 column. Tartrate was not detected in any of the açaí samples, but it was one of the dominant components in grape juice (see Figures 1−3), at a concentration of 1.92 mg/g.

Concentrations of all the anions, determined using the AS24 column, are listed in Table 1. The calibration plots for these analyte ions are given in the Supporting Information in Figures S5−S8. The calibration ranged from 0.2 μM at the low end to 100 μM at the high end, depending on the analyte. The calibration equations are listed in Table S2 in the Supporting Information for the stated ranges; even for the weak acids, at the low concentrations examined, the peak area response was acceptably linear. The linear determination coefficient (r^2) ranged from 0.9914 for hexanoate to 0.9996 for phytate; these are listed in the table. Also given therein are the limits of detection (LOD) for each ion under these chromatographic conditions based on the S/N = 3 criterion; these ranged from 3 nM for the hexaprotic acid anion phytate to 200 nM for the monoprotic weak acid anion hexanoate.

There is no particular column that is uniquely better than all others to perform juice/extract analysis either for authentication or to differentiate between different strains of açaí. Whereas AS24 can separate most anions, it cannot separate tartrate and oxalate. The latter separation is facile on AS15, but that column cannot separate early-eluting ions, for example, acetate, formate, and lactate or elute phytate in an hour. Thus, quantitation of all but oxalate and tartrate was done on the AS24 column, whereas oxalate and tartrate were separated and quantitated on the AS15 (see Figure S5 in the Supporting Information).

Mass Spectrometric Identification of Galacturonate, Hexanoate, and Phytate. Some peaks were not initially identifiable on the basis of known chromatographic retention behavior. The gradient described for the AS24 column was stretched out some over time to better elucidate the nature of these peaks. The chromatogram of the Florida açaí extract under these conditions is shown in Figure 4. The virtually coeluting peaks at 21 min revealed, on negative ion mode mass spectrometric selected ion monitoring study, ions at 115 and 193 Th. The 193 Th signal was ascribed to galacturonate. The retention time and the fragmentation pattern matched exactly with a standard.

The 115 Th response was ascribed to hexanoate. A hexanoate standard was used for confirmation; the retention time was within 0.02 min of that in the sample, which is well within the reproducibility of retention times under these conditions. The hexanoate concentration in the sample was too low to quantitatively compare the relative peak intensities in the fragmentation pattern in the sample and the standard. However, the Th values of all the fragment peaks were identical in the sample and the standard, within the mass resolution of the instrument. The relevant data are shown in Figures S9 and S10 in the Supporting Information.

The peak that eluted after citrate at ~52 min was also initially not identified. This peak produced an intense signal at 219 Th. The peak was asymmetric and tailed into the higher Th range, often a characteristic of multiply charged ions that fragment into a less charged ion at a higher Th. Reinvestigation of the full spectrum scan showed the presence of a weaker signal at 329 Th. Both signals at 219 and 329 Th would be consistent with

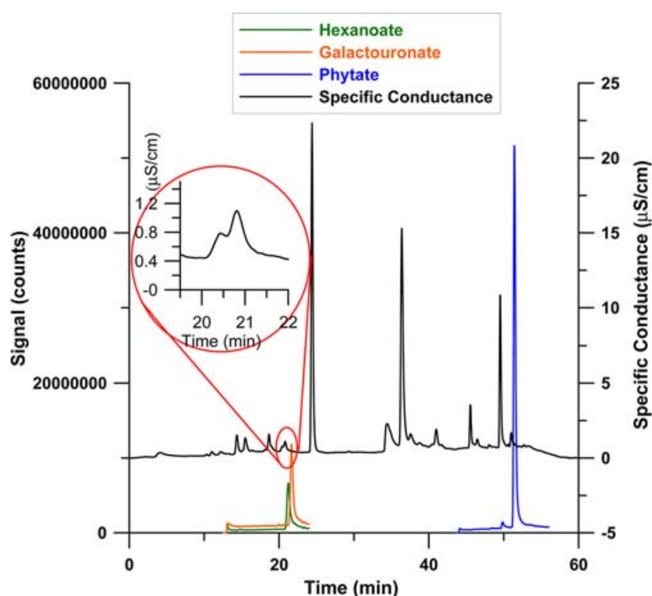

Figure 4. Chromatogram of Florida açaí on AG24-AS24 column set, at a slightly slower gradient relative to Figure 1. (Black trace) Right ordinate, conductivity detector. Hexanoate and galacturonate elute in the 20−22 min window, and phytate elutes around 52 min. The selected ion monitoring counts (left ordinate) and the corresponding traces (green, red, blue) are shown. See text for details.

being derived from a neutral species of 660 mass units: the above two signals resulting respectively from the loss of three and two protons. As the singly charged species was not observed, we suspected the parent compound to be a multiprotic strong acid. Higher resolution spectra were used for confirmation and are shown in the Supporting Information. Note that a clear isotope distribution, separated by ∼0.33 Th, is observed for the 219 Th peak (Supporting Information, Figure S11), indicating a triply charged ion. Fragmentation was performed from 30 to 218 Th and from 220 to 659 Th; the results are shown in the Supporting Information (Figure S12). The parent mass and isotope distribution suggested a possible chemical formula of $C_6P_6O_{24}H_{18}$. Nominal Th values of all of the major ions seen are consistent with fragments that will logically be generated from the triply charged phytate ion (Supporting Information, Table S3). Additionally, given that the chromatographic retention time matched exactly with an authentic standard of phytate (*myo*-inositol hexakisphosphate), we positively identified this chromatographic peak as phytate. Table 2 contains a list of all major fragments ions listed in the order of their intensity along with their origin.

Significance of Phytic Acid. The structure of phytic acid is shown in the Supporting Information (Figure S13). It is found in soil, cereals, legumes, nuts, oil seeds, pollen, and spores.[46] Reported health effects of phytic acid are somewhat contradictory. Whereas it has been called an antinutrient for several decades (it removes metals such as calcium as water-insoluble complexes, preventing absorption in the gut), it is now known that it also acts as an antioxidant and anticancer agent.[47] Phytate may also help prevent Alzheimer's disease and other neurodegenerative diseases by chelating iron (it has a greater affinity for iron than calcium) and keeping it from initiating oxidative damage.[48] The main sources of phytate in the daily diet are cereals and legumes, especially oily seeds and nuts, with an estimated daily intake in the West ranging between 0.3 and 2.6 g.[47] The recommended daily dosage for açaí powder formulated as capsules and taken as supplement is 2 g/day. The Florida açaí contained a much greater amount of phytate (∼6 times) than the Brazilian açaí. However, on an absolute scale, the amount of phytate in the Florida sample was only 1.34 mg/g; it will not be a significant source for phytate. On the other hand, it is possible that Florida and Brazilian açaí can be distinguished by their phytate content.

Table 2. Putative Ion Identification of Parent and Daughter Ions of the Triply Charged Phytate Peak

m/*z* (Th)	ion	loss
219	$C_6P_6O_{24}H_{15}^{3-}$	*parent ion*
289	$C_6P_5O_{21}H_{15}^{2-}$	PO_3^-
499	$C_6P_4O_{18}H_{15}^-$	$2*PO_3^-$
401	$C_6P_3O_{14}H_{12}^-$	$2*PO_3^- + HPO_3^- + H_2O$
79	PO_3^-	ejected ion
481	$C_6P_4O_{17}H_{13}^-$	$2*PO_3^- + H_2O$
321	$C_6P_2O_{11}H_{11}^-$	$2*PO_3^- + 2HPO_3^- + H_2O$
303	$C_6P_2O_{10}H_9^-$	$2*PO_3^- + 2HPO_3^- + 2H_2O$
240	$C_6P_4O_{17}H_{12}^{2-}$	$PO_3^- + HPO_3^- + H_2O$
383	$C_6P_3O_{13}H_{10}^-$	$2*PO_3^- + HPO_3^- + 2H_2O$
419	$C_6P_3O_{15}H_{14}^-$	$2*PO_3^- + HPO_3^-$
177	$H_3P_2O_7^-$	possible gas phase recombination
159	$HP_2O_6^-$	possible gas phase recombination

Implications on Phytate Analysis. The analysis of phytate is important, and the present method provides a superior alternative to extant methods. AOAC method 986.11[49] uses anion exchange separation and then complete hydrolysis and colorimetric phosphate determination. If other inositol phosphates are not completely separated, a positive error results.

In SCAC (obligatorily with an alkaline eluent), phytate will have a charge of −12. The normal expectation will be that it will not elute in a reasonable period, as was presently observed with the AS15 column. Indeed, in previous studies[50] even to elute the triphosphate from an AS4A column required high concentrations of 4-cyanophenolate, an extremely strong eluent. Only the mono- and diphosphate could be eluted with a high concentration gradient of NaOH and NaOAc from a PA-10 column.[51] Strong acid eluents with anion exchange columns and post column reactions with Fe^{3+} can separate and detect (by UV absorption) all inositol phosphates.[52−54] However, unlike the present method, it will not sensitively detect other ions, for example, organic acids, or be compatible with mass spectrometry.

The present method can elute phosphate in a reasonable period with a hydroxide eluent. If phytate is the only inositol phosphate analyte of interest, we have found that it can be eluted much sooner using a stronger eluent gradient but without causing coelution with the peaks eluting earlier.

Implications on the Authentication of Açaí. There is considerable interest in authentication of expensive foods and beverages that can be easily adulterated. For example, for pomegranate juice, an international multidimensional authenticity specification algorithm has recently been established.[10] The present work suggests on a preliminary basis that the absence of tartrate may provide a useful tool to determine the authenticity of pure açaí and/or pomegranate products, especially as the common adulterant juices are all known to contain large to easily detectable levels of tartrate. Of course, many more samples would have to be analyzed to establish the

validity of this observation. It is important that any such analysis be done on genuine açaí. For example, it was recently reported that a product labeled "açaí juice" contained 0.6 mg/L nitrate.[9] Nitrate is unlikely to be present inside the fruit, and no nitrate was found in any of the açaí samples in the present study. The ability to differentiate between Florida and Brazilian açaí on the basis of phytate content will also need to be validated by analysis of a much larger number of samples.

The measurement of principal organic acids in fruit juices, especially grape juice (for general characterization and for determination of maturity for harvesting), by SCAC has been previously advocated.[55−57] The SCAC technique has grown considerably in power since; presently, it is possible to generate an essentially complete anion profile, including that of the organic acids, even those present at low levels. The different selectivities of the multitude of columns presently available permits complete separation and also aids identification through multidimensional retention mapping as shown in Figure 5. For example, lactate elutes before acetate on an AS24

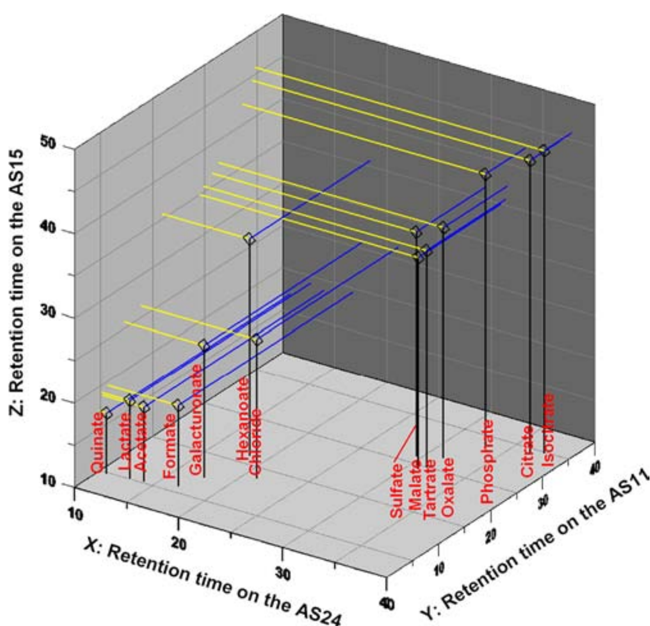

Figure 5. Three-dimensional retention map of different anions on the AS24, AS15, and AS11 columns.

column, whereas the reverse elution order is observed on an AS15. Similarly, the elution orders of sulfate and malate are reversed between AS24 and AS11, among many such instances. In most cases, the use of mass spectrometry is not essential. With the açaí products as the focus, we hope to have clearly established the power of this technique.

■ ASSOCIATED CONTENT

Supporting Information

Gradient program used for different columns, chromatograms on an AS15 column at different concentration levels, chromatograms on an AS11 column, chromatograms on an AS24 column in the 8−22 min region, calibration plots for various ions, calibration parameters and detection limits for various ions, mass fragmentation pattern of the 115 Th peak and comparison with a hexanoic acid standard, mass fragmentation pattern of the 219 Th peak putative identification of the fragmentation tree, and structure of phytic acid. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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