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Isotopes as Tracers of the Hawaiian Coffee-Producing Regions

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Supporting Information

ABSTRACT: Green coffee bean isotopes have been used to trace the effects of different climatic and geological characteristics associated with the Hawaii islands. Isotope ratio mass spectrometry (IRMS) and inductively coupled plasma mass spectrometry ((MC)-ICP-SFMS and ICP-QMS) were applied to determine the isotopic composition of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), sulfur ($\delta^{34}S$), and oxygen ($\delta^{18}O$), the isotope abundance of strontium ($^{87}Sr/^{86}Sr$), and the concentrations of 30 different elements in 47 green coffees. The coffees were produced in five Hawaii regions: Hawaii, Kauai, Maui, Molokai, and Oahu. Results indicate that coffee plant seed isotopes reflect interactions between the coffee plant and the local environment. Accordingly, the obtained analytical fingerprinting could be used to discriminate between the different Hawaii regions studied.

KEYWORDS: isotopes, coffee, multielement analysis, IRMS, ICP-MS

INTRODUCTION

Several studies have shown that green coffee beans from different geographical origins have different elemental and isotopic compositions.[1,2] Krivan and collaborators[1] demonstrated the potential of measuring elemental fingerprints of coffee beans to discriminate between different origins. This study was complemented by Serra and coauthors[2] with the determination of the isotopic composition of carbon, nitrogen, and boron in green coffees from 19 different countries, showing that the isotopic composition of these three elements is a good indicator of geographical-dependent parameters and, therefore, a useful tool to infer the region of production of green coffee. However, the study of the relationships between isotopes of the coffee plant seed and environmental factors is still recent.[3] Rodrigues and coauthors[3] have determined isotope ratios of carbon, nitrogen, oxygen, and strontium of green coffee beans and have searched for relationships between the measured isotope ratios and available information on environmental factors. Such studies are important to the understanding of how the seed integrates isotope fractionations occurring during its development, associated with change of local climate and geology. This may ultimately lead to the discrimination of coffee-producing regions. Currently, stable isotope analysis is a powerful tool in ecological studies to trace, record, source, and integrate ecological parameters of interest and has been extensively used in food authentication studies.[4] The H, C, N, O, S, and Sr isotopes are the elements that vary the most on Earth, that constitute the bulk of all living matter, and that are used most effectively to track changes in the Earth's biogeochemical cycles. With regard to carbon isotopes, the basis for much of the observed variation in $\delta^{13}C$ of organic samples derives from two metabolic processes, photosynthesis and respiration.[5] Also, as the environment changes, a wide range of $\delta^{13}C$ values within biological materials suggests multiple and very different processes leading to this observation, such as stomatal control.[6] In relation to oxygen, meteoric waters can vary in their $\delta^{2}H$ and $\delta^{18}O$ as they move through the hydrological cycle.[7] Many important factors influence the $\delta^{2}H$ and $\delta^{18}O$ of meteoric waters, for example, seasonality and therefore the changes in condensation temperatures of precipitation, latitude, altitude, and orographic barriers.[8] Once water is taken up by plants, the $\delta^{2}H$ and $\delta^{18}O$ of "body/source" water and biosynthetic compounds that incorporate H or O may or may not also have associated fractionations that are "recorded" in the organic molecules that contain these elements.[9,10] As the processes involved in these fractionations become better understood,

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plant materials present themselves as valuable "biomarkers" of ecological processes because when tissues are formed, they are known to record temperatures, water sources, and levels of relative humidity prevailing at that time.[11] In the case of nitrogen, variation in the $\delta^{15}N$ in its cycle processes has been increasingly studied. Knowledge of how the isotopes of N fractionate during catabolic reactions in soils and in plants in relation to N utilization, transformation, and fixation elucidate the pathways and interactions that many times result from land-use and agricultural practices.[12] In addition, it has become apparent that the use of both sulfur (S) and strontium (Sr) isotopes holds great promise for detecting and therefore understanding the nature and magnitude of ecological change. Sulfur stable isotopes have been useful in pollution studies.[13,14] For all Sr isotope research, it is important to understand that the $^{87}Sr/^{86}Sr$ ratio depends on what the parent−daughter rubidium (Rb) to strontium ratio (specifically, $^{87}Rb/^{86}Sr$) in the source is and how long ago in time it fractionated. Although isotope fractionation in seeds (e.g., coffee beans) is yet poorly understood, previous work suggests that the coffee bean may be a valuable indicator of ecological processes and geology.[3] Nonetheless, a solid interpretation of isotope abundance in the green coffee bean is complicated by the combination of environmental, climatic, and physiological processes. The results obtained so far suggest that there is not a unique interpretation for the distribution of isotopic composition of green coffee beans at global scale. Seasonal variations in humidity, temperature, and precipitation, as well as geology, and experimental or human impacts influence the isotopic signatures (ratios) of elements in coffee. These effects make data interpretation more challenging but may enable the discrimination of easily delineated small regions. For this reason, a scale-down was done in this study of coffees produced in the state of Hawaii, the gourmet quality of which is known worldwide.

The goal of this work was to measure the isotopic composition (C, N, O, S, and Sr) and multielemental concentrations in green coffees to differentiate between Hawaiian coffee-producing regions. To interpret isotope variations in the Hawaiian green coffee beans, relationships between results obtained and available information on altitude, volcanic activity, and annual mean $\delta^{18}O$ values of precipitation were also addressed.

MATERIALS AND METHODS

Samples and Climate. Green Arabica coffee beans (47 samples) from five different Hawaii coffee-producing regions were provided by Coffea Consulting and the University of Hawaii. The Hawaii state regions included in this study were Hawaii, Kauai, Maui, Molokai, and Oahu (Figure 1). Hawaii was the only region where it was possible to collect samples from two different harvest years, 2007 and 2008. All other green coffee bean samples included in this study dated from 2007. The samples from Hawaii were obtained from three districts: Kau, Puna, and Kona (Figure 1), where farms are characterized for being just a few square meters. The harvest period in the Hawaii region starts in June/August (depending on altitude) and extends until February/March, the middle point being September/October. Each sample from the Kau district was collected at a different farm. The 13 coffee samples from the Kona district were obtained from eight farms. In five of these eight farms, it was possible to obtain one sample from 2007 and one from 2008. The two samples from Puna were obtained from two different farms, during the harvest period of 2007. Samples from Eleele at Kauai (Figure 1) originated from a single estate farm considered to be the largest in Hawaii state, with more than 12000 m^2 of coffee in production. One sample was obtained also at this region but from another farm at Kapahi. In Kauai, the coffee harvest period is shorter compared to the other regions, beginning in late August and ending in late November. In the case of Maui, Molokai, and Oahu, coffee is harvested from July/August until February. Samples from Maui were obtained from a farm located in Kaanapali and from another farm in Kula (Figure 1). The two samples from the Molokai region were produced from the same farm. In the Oahu region, samples were collected from a farm at Waialua and from farms at Waiahole and Kunia (Figure 1). Each green coffee bean sample consisted of 100 g of green coffee beans. The samples were packed under vacuum and transferred to the laboratory for further analysis. Whenever possible, latitude and longitude data were obtained with Google Earth software, version 5.0,[15], and annual mean $\delta^{18}O_{prec}$ was acquired from the Online Isotopes in Precipitation Calculator (OIPC 2.2).[16] Information on temperature (daily mean values for each geographical location) was acquired from the Hawaii Natural Resource Information System (HNRIS). [17]

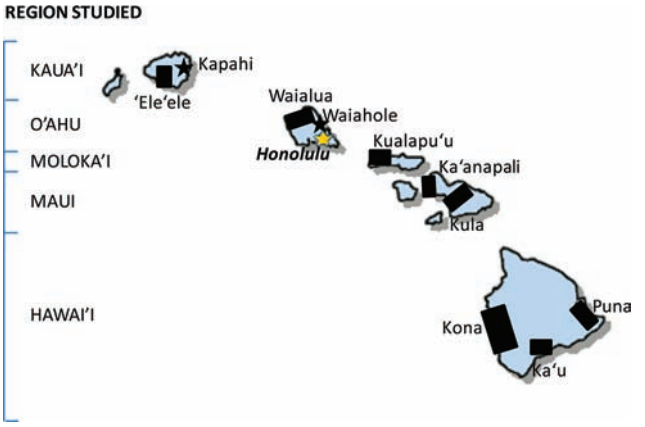

Figure 1. Different Hawaii regions and corresponding districts (dark rectangles; approximate location) from where green coffee bean samples were obtained.

Isotope Ratio Mass Spectrometry (IRMS). *Sample Preparation.* Each green coffee bean sample (100 g) was ground in a mill (Type MM2, Retsch, Germany), three times for 5 min each time, to obtain particle sizes of <1 mm to achieve a homogeneous sample. After grinding, samples were dried overnight at 45 °C and then weighed in tin capsules that were then folded close. Moreover, from one of the samples (chosen randomly), 30 green coffee beans were separated and ground individually. The goal was to analyze the 30 green coffee beans separately for C, N, S, and O isotopic composition to have an indication of the standard deviation of the isotopic composition of each element within each coffee.

Combustion (EA-C) mode. Carbon stable isotope ratio was determined on a SIRA II (VG Isogas, U.K.) stable isotope ratio mass spectrometer coupled to an elemental analyzer (EuroVector, Italy) for sample preparation by combustion−reduction. Nitrogen and sulfur stable isotope ratios were determined on an Isoprime (Micromass, U.K.) isotope ratio mass spectrometer coupled to an elemental analyzer (EuroVector). Coupling of the elemental analyzers and isotope ratio mass spectrometers was via open split. Sulfur isotope ratios were determined by Dumas combustion/reduction at 1025 °C on a quartz reactor with tungsten oxide on alumina as oxidation catalyst and pure reduced copper wires as reduction agent for removal of excess oxygen. Water resulting from combustion was removed with a magnesium perchlorate trap, and gas separation was achieved on a gas chromatography column for S (EuroVector), maintained at 95 °C. Isotope ratios were calibrated against international standards, namely, IAEA CH6 (sucrose) and IAEA CH7 (polyethylene) for carbon isotope ratio, IAEA N1 (ammonium sulfate) for nitrogen isotope ratio, and IAEA S1 (silver sulfide) and

NBS 127 (barium sulfate) for sulfur isotope ratio. Analytical performance, stability and drift, was checked by inserting laboratory standards between samples, that is, sorghum flour standard OAS (B2158, Elemental Microanalysis) for carbon and nitrogen and ground green coffee bean for sulfur. Correction was made when necessary. Precision (standard deviation of the set of standards analyzed in each batch, $n = 6$) was 0.06‰ for carbon, 0.08‰ for nitrogen, and 0.3‰ for sulfur isotope ratio determinations. Carbon, nitrogen, and sulfur isotopic compositions of 30 individual coffee beans from a single site were determined to estimate the possible variation of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ within each coffee sample. The standard deviations obtained for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of the 30 individual beans were 1.4, 0.8, and 0.5‰, respectively. The histograms of the results obtained for the determination of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of the 30 individual coffee beans of the same coffee are shown in Figure S1 of the Supporting Information.

Pyrolysis (EA-P) Mode. Oxygen isotope ratios were determined on an Isoprime isotope ratio mass spectrometer coupled to an elemental analyzer (EuroVector) by high-temperature pyrolysis. Pyrolysis was accomplished at 1300 °C on a glassy carbon reactor with glassy carbon chips and nickel-plated carbon as catalysts, mounted coaxially on a ceramic tube. Coupling of the elemental analyzer and isotope ratio mass spectrometer was performed via open split. The isotope ratio data were corrected against international standards (IAEA 601 and IAEA 602). Analytical performance, stability and drift, was checked by inserting laboratory standards between samples. Correction was made when necessary. Precision was 0.14‰. Oxygen isotopic composition of 30 individual coffee beans yielded a standard deviation for $\delta^{18}O$ of 0.4‰.

Strontium Isotope Ratio Measurement by Multicollector Inductively Coupled Plasma Sector Field Mass Spectrometry (MC-ICP-SFMS). *Reagents.* Pro analysis (p.a.) grade 65% HNO_3 (Merck, Darmstadt, Germany) was subboiled doubly in an ultrapure quartz apparatus (MLS DuoPur, MLS, Leutkirch im Allgäu, Germany). Deionized water (18 MΩ cm; SG, Wasseraufbereitung and Regenierstation GmbH, Barsbuttel, Germany) was subboiled prior to usage as well. Subboiled HNO_3 and 31% H_2O_2 (p.a. grade, Merck) were used for microwave-assisted digestion. Polyethylene flasks and cartridges as well as polypropylene tubes and lids were cleaned sequentially with HNO_3 (10% (v/v)) and HNO_3 (1% (v/v)) and rinsed with deionized water before use. Dilution of standards and samples was performed gravimetrically with HNO_3 (1% (v/v)), prepared from subboiled water and doubly subboiled HNO_3. A 20 ng g^{-1} solution of SRM 987 $SrCO_3$ (NIST, Gaithersburg, MD) was used for quality control of the Sr isotope ratio measurements. The certified $^{87}Sr/^{86}Sr$ ratio is 0.71034 ± 0.00026, whereas a generally "accepted value" of the $^{87}Sr/^{86}Sr$ ratio for this reference material is reported in the literature as 0.710263 ± 0.000016 (the error represents a range of 2 standard deviations determined from the external reproducibility).[18]

Sample Preparation. Four to six beans (amounting to about 1.0 g) were ground in a Retsch mill type MM2, three times for 5 min each time, to obtain particle sizes of <1 mm. Approximately 0.5 g of the ground material was directly weighed into Teflon bombs for subsequent microwave-assisted digestion (MLS 1200mega, MLS). Concentrated double-subboiled HNO_3 (6 mL) and H_2O_2 (1 mL) were used as digestion reagents. Details are presented elsewhere.[19] The samples were finally transferred into 50 mL flasks, filled with HNO_3 (1% v/v to 20 g, filtered using a 5 mL syringe through 0.45 μm filters (Minisart RC 25) and stored at room temperature for future analysis. A digestion blank was prepared with each digestion batch.

Strontium/Matrix Separation. The obtained digestion solutions of green coffee bean samples were separated according to the method of Swoboda and coauthors,[19] using Eichrom Sr resin (Eichrom Industries, Darien, IL). The solutions were diluted after separation to a final Sr concentration of about 20 ng g^{-1} to obtain optimum signal intensities of ^{88}Sr from 3 to 5 V. The fractionation effect of the column extraction procedure on the Sr isotopic ratio was checked according to the same authors[19] and proved to be insignificant.

Instrumentation. Screening of the solutions for Rb and Sr prior to and after separation was performed by using a quadrupole-based inductively coupled plasma mass spectrometer ICP-MS (Elan DRCe, Perkin-Elmer, Waltham, MA). Sr isotope ratio measurements of the final solutions were accomplished using a double-focusing multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS) (Nu Plasma HR, Nu Instruments Ltd., Wrexham, U.K.) coupled to a membrane desolvating system (DSN 100, Nu Instruments Ltd.). The DSN 100 instrument was equipped with a PFA nebulizer (MicroFlow nebulizer, Elemental Scientific, Omaha, NE) and a spray chamber with additional hot gas flow to eliminate condensation and droplet formation. The multicollector inductively coupled plasma mass spectrometer is equipped with a collector configuration consisting of 12 Faraday cups and 3 ion counters. The latter were not used throughout this study. All isotopes in this work (^{82}Kr, ^{83}Kr, ^{84}Sr, ^{85}Rb, ^{86}Sr, ^{87}Sr, and ^{88}Sr) were measured simultaneously using Faraday cups. Experimental parameters of the MC-ICP-MS including nebulizer gas, rf power, and ion transfer lens potentials were optimized to achieve the maximum ion intensity for ^{88}Sr, using NIST SRM 987 solution at a concentration of 20 ng g^{-1}. The operation parameters are described in detail in the Supporting Information (Table S1). Blank correction and mass bias correction were performed according to previous measurements.[19]

Multielement Analysis by ICP-MS. All digestion solutions obtained for Sr isotope analysis were screened for different elements, and their total concentration was calculated through external calibration performed with a multielement standard solution VI (Merck KGaA). Nine calibration levels were prepared with concentrations of 0.05, 0.1, 0.5, 1, 5, 10, 25, 50, and 100 ng g^{-1}, respectively. For internal normalization, an indium standard was added to a final content of 10 ng g^{-1}. HNO_3 (1% (v/v)) was used for blank correction. The blank corrected concentrations of the different isotopes in each sample were calculated by the instrumental software through external calibration, based on linear regression and/or weighted regression and internal normalization after blank subtraction. The determination of the element concentrations was accomplished with a quadrupole mass spectrometer ELAN DRC-e (PerkinElmer, Ontario, Canada) and, in the case of rare earth elements (REE), with a high-resolution sector field mass spectrometer ELEMENT 2 (Thermo Scientific, Bremen, Germany) under the operational conditions described in Table S2 of the Supporting Information. The concentrations of the elements B, Na, Mg, Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Rb, Sr, Mo, Ba, Pb, Bi, Y, La, Ce, Pr, Sm, Nd, Eu, Dy, Th, Sc, Ho, and Gd were determined in all green coffee bean samples. The uncertainty of the multielement measurements was calculated using GUM Workbench Pro (version 1.2, Metrodata GmbH, Germany). The Guide to the Expression of Uncertainty in Measurement (GUM) was published by ISO and establishes the general rules for evaluating and expressing uncertainty.[20] The limit of detection (LOD) was calculated according to the method of Thomsen and coauthors.[21]

Statistical Analysis. Multivariate statistics for the classification of coffee samples of various origins was performed in SPSS version 15.0. A canonical discriminant analysis including a leave-one-out classification (U method) was performed, and the classification score was re-examined via cross-validation. The variance expressed in the eigenvalue was set to be >1 for both methods. One-way ANOVA analysis was used to evaluate significant differences among coffee-producing regions with regard to each element analyzed in this study and was performed with the Statistica software (version 9.0) (Statsoft, Tulsa, OK).

RESULTS AND DISCUSSION

Coffee Bean Oxygen Isotopic Composition. The oxygen isotopic composition of the green coffee beans samples varied

Table 1. Origin, Daily Mean Temperature, Annual Mean $\delta^{18}O$ of Precipitation, and Isotopic Composition of C, N, O, S, and Sr of Hawaiian Green Coffees (Whenever $n \geq 3$, Average and Standard Deviation Are Shown)

island	specific location	altitude[a] (m)	temperature[b] (daily mean; °C)	annual mean $\delta^{18}O$ of precipitation[c] (‰)	$\delta^{18}O$ (‰)	$\delta^{34}S$ (‰)	$^{87}Sr/^{86}Sr$	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Hawaii	Kau	<400 (n = 1)	22.2	−3.0	27.8	0.4	0.7051	−27.4	2.3
		400−500 (n = 3)	21.9	−3.2	27.9 (0.2)	3.3 (3.0)	0.705 (0.0002)	−24.4 (2.0)	0.8 (0.5)
		500−600 (n = 2)	22.2	−3.4	28.3	3.5	0.7051	−24	4.4
					28.8	0.8	0.7051	−26.4	1.2
		700−750 (n = 2)	17.9	−3.7	28.5	1.6	0.7052	−25.7	0.7
					28.9	−1.5	0.705	−25.2	1.2
	Kona	200−400 (n = 2)	23.4	−2.7	25.9	3.5	0.7062	−26.9	1.3
					27.1	6.4	0.7070	−25	1.5
		400−600 (n = 5)	22.6 (1.4)	−3.3 (0.1)	28.1 (0.7)	7.7 (2.7)	0.7057 (0.0008)	−25.4 (0.9)	1.9 (0.7)
		700−800 (n = 6)	21 (1.5)	−3.8 (0.1)	26.6 (1.2)	3.8 (1.4)	0.7067 (0.001)	−26.5 (2.5)	1.9 (0.3)
	Puna	30 (n = 1)	22.6	−2.3	26.4	5.7	0.7084	−27.6	3.3
		400 (n = 1)	20.6	−3.1	26.8	12.7	0.7073	−24.3	1.3
Kauai	Eleele	<150 (n = 8)	–[d]	−2.7 (0.04)	31.5 (0.6)	18 (1.8)	0.7057 (0.0006)	−27.1 (1.1)	1.9 (0.3)
	Kapahi	50 (n = 1)	23.2	−2.6	30.2	10.5	0.7067	−26.2	2
Maui	Kāanapali	100−150 (n = 6)	22.5 (0.3)	−2.7 (0.1)	30.4 (1.1)	16.8 (3.8)	0.7071 (0.0002)	−27.6 (0.6)	1.3 (0.1)
	Kula	1000 (n = 1)	17.2	−4.3	28.3	11.1	0.7063	−30.6	0.9
Molokai	Kualapuu	250 (n = 2)	22.6	−2.9	29	9.8	0.7086	−28.7	5.4
					31.1	9.7	0.7082	−29.3	2.1
Oahu	Waialua	<250 (n = 4)	22.7 (0.3)	−2.8 (0.1)	30.6 (2.2)	10.4 (2.7)	0.7087 (0.0002)	−27.2 (1.8)	1.9 (0.7)
	Waiahole	50 (n = 1)	23.6	−2.6	29.2	15.2	0.7091	−25.2	3.8
	Kuna	80 (n = 1)	–	−2.6	32.5	14.4	0.7056	−27.5	1.2

[a] Latitude and longitude values are not shown to maintain grower or farm anonymous. [b] From HNRIS.[17] [c] From OIPC.[16] [d] Data are unavailable for the selected location.

by 7‰. The $\delta^{18}O$ values of the different coffees varied from 25.9‰ (Hawaii) to 32.5‰ (Oahu; Table 1). The analysis of 30 individual coffee beans from the same coffee sample showed a standard deviation of 0.4‰. This value was assumed as indicative of the variation of the isotopic composition of oxygen within each coffee sample. The values measured for each individual green coffee bean sample included in this study are shown in Table S3 of the Supporting Information. Higher values of mean $\delta^{18}O$ were obtained for coffees produced at lower altitude. All coffees with $\delta^{18}O$ >29‰ were produced at altitudes of <300 m (Figure 2). Coffees from Eleele and Kapahi (Kauai), from Kunia, Waialua, and Waiahole (Oahu), from Kualapuu (Molokai), and from Kaanapali (Maui), produced at altitudes of <250 m, had a mean $\delta^{18}O$ value from 29 to 32.5‰ (Table 1). In comparison to these sites, coffees from the Hawaii region, and Kula in Maui, had lower mean $\delta^{18}O$ values, ranging from 25.9 to 28.9‰ (Table 1). The mean $\delta^{18}O$ values per region, and respective standard deviations, are shown in Figure 3a. This allowed the separation of two groups: Hawaii and other regions. For each coffee, known values of latitude, longitude, and altitude allowed predicted values of $\delta^{18}O$ of local precipitation to be obtained with the OIPC (The Online Isotopes in Precipitation Calculator).[16] A positive correlation was obtained between the $\delta^{18}O$ of the coffee beans and of local precipitation (r = 0.56; p < 0.05). These results indicate that the isotopic composition of oxygen of the Hawaiian coffees studied varies according to the altitude at which they are

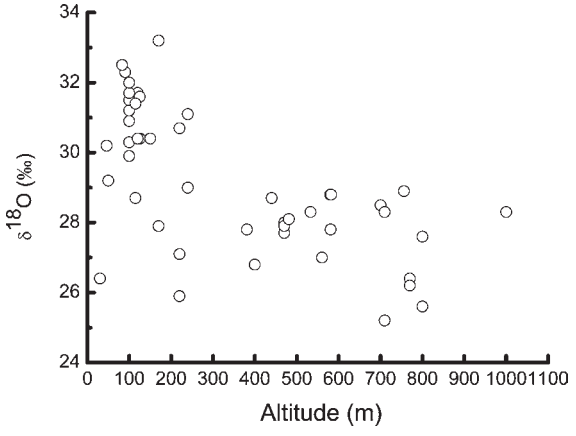

Figure 2. $\delta^{18}O$ of the green coffee beans in relation to altitude.

produced, a parameter that is known to influence the isotopic composition of local precipitation (due to changes in temperature). Although the isotope fractionation processes occurring during the coffee plant fruit and seed development are still poorly understood, the isotopic composition of the coffee bean is expected to be the result of the fractionations associated with several metabolic pathways of the plant, occurring during the seed development period (8 months on average in the case of

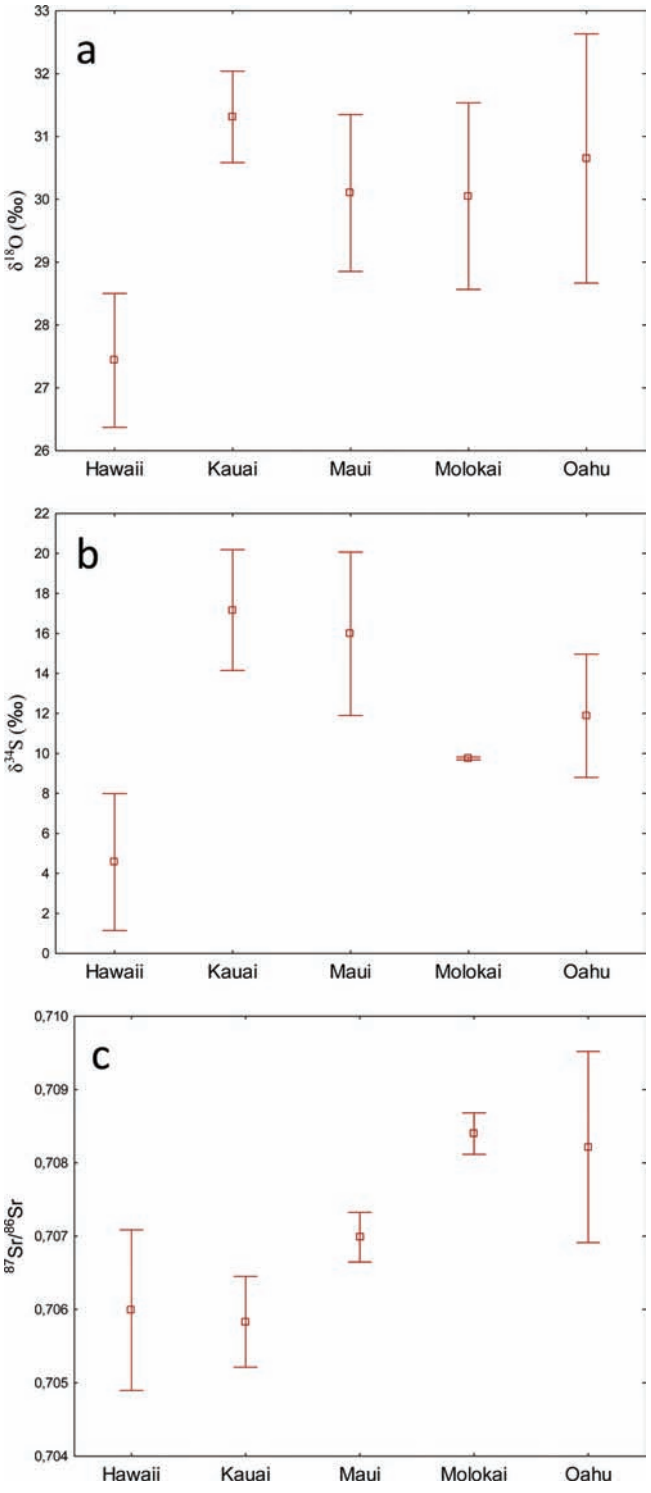

Figure 3. $\delta^{18}O$ (a), $\delta^{34}S$ (b), and $^{87}Sr/^{86}Sr$ (c) mean ± SD values of green coffee bean samples from Hawaii (n = 24), Kauai (n = 9), Maui (n = 7), and Oahu (n = 6). (Molokai was not included as n = 2.)

Coffea arabica). Several authors have shown that the $\delta^{18}O$ of water in plants changes as a result of variations in $\delta^{18}O$ of water taken up by the plants and leaf water enrichment of ^{18}O during transpiration, the latter of which is dependent on atmospheric conditions (relative humidity and $\delta^{18}O$ of water vapor in the atmosphere), of stomatal regulation of water loss, and of the variation in $\delta^{18}O$ of water in cells forming organic material.[6,22] This supports the results observed in this work and the relationship between the $\delta^{18}O$ values of the green coffee beans and of local precipitation, with variations possibly associated with each region's characteristic climate and plant ecophysiology. Coffees from the Hawaii region are produced at higher altitudes in relation to the other regions, which, in turn, can be associated with more depleted $\delta^{18}O$ of precipitation (Table 1). On this island, higher values of coffee bean $\delta^{18}O$ were observed only at low altitude, where higher $\delta^{18}O$ of precipitation is expected. However, some of the more depleted coffee $\delta^{18}O$ values come from lower altitude, as in the case of the coffees from the Kona region (Table 1). This area receives rainfall from storm systems unrelated to trade winds, which can be accentuated by frontal systems and "Kona storms".[23] As a consequence, more depleted $\delta^{18}O$ values of rainfall have been observed. This difference in the $\delta^{18}O$ of storm precipitation coincides with the results observed for $\delta^{18}O$ values measured in the Kona and Puna coffees, despite the lower altitude.

Coffee Bean Sulfur Isotopic Composition. The variation observed in the sulfur isotopic composition of the different coffees was 20‰. Values of $\delta^{34}S$ of the green coffee bean samples ranged from −1.5 to 21.3‰ (Table 1). The standard deviation of $\delta^{34}S$ determined for 30 individual coffee beans from the same coffee sample was 0.5‰. Coffees from the Hawaii region had $\delta^{34}S$ values from −1.5 to 7.7‰, with the exceptions of a sample from Kona (11.9‰) and one from Puna (12.7‰) (Table 1). The $\delta^{34}S$ values of Hawaii coffees were lower than those from Kauai, especially in the case of Eleele, which showed a $\delta^{34}S$ average of 18 ± 1.8‰ (Table 1). Also in Kauai in Kapahi, the observed $\delta^{34}S$ value was 10.5‰. Coffees from Maui showed $\delta^{34}S$ of 16.8 ± 3.8‰ in Kaanapali (Table 1). In the case of Molokai, the observed $\delta^{34}S$ values of the two coffee bean samples were 9.8 and 9.7‰ (Table 1). In Oahu, $\delta^{34}S$ varied from an average of 10.4 ± 2.7‰ (Waialua) to values of 14.4 and 15.2‰ at Kuna and Waiahole, respectively (Table 1). Despite the wide range of coffee $\delta^{34}S$ values observed, it is possible to differentiate coffees from the Hawaii region from those produced on other islands (Figure 3b). The higher $\delta^{34}S$ values (>15‰) were observed at altitudes of <200 m, in locations closer to the ocean, which in most cases correspond to regions other than Hawaii (Table 1). For most plants, the normal source of sulfur is the sulfate taken up by the soil fine roots. The plant's assimilatory sulfate reduction that provides "organic sulfur" from sulfate proceeds without important sulfur isotope fractionations.[24] In general, bulk plant sulfur is depleted by only 1−2‰ relative to its primary sources, soil and sea spray sulfates or SO_2 from the atmosphere.[24] Although the details of sulfur isotope biochemistry in coffee plant seeds are unknown, it is not unreasonable to hypothesize that green coffee beans record the isotopic signature of the sulfur source(s). One possibility could be the use of fertilizers, which also influence $\delta^{34}S$ of sulfates in soil and plants. Several coffees included in this study were produced with organic fertilizers, whereas others were grown with the application of synthetic fertilizers. However, differences between organic and synthetically fertilized coffees with regard to coffee bean $\delta^{34}S$ have not been observed. The presence of active volcanos in some of the regions included in this study is also expected to influence the $\delta^{34}S$ values of the coffee bean samples. In the Hawaiian islands, $\delta^{34}S$ values of sulfates from volcanic ash and basalt-derived soils ranging from 6.3 to 18‰ have been reported.[25] Most importantly, the more enriched of those values correspond to shorter distances to the sea.[25] Our results

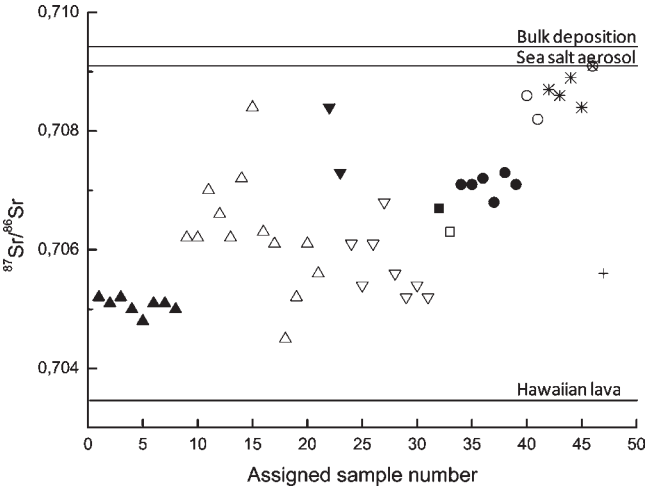

Figure 4. Sr isotope ratio of green coffees from Hawaii: (▲) Kau (Hawaii); (△) Kona (Hawaii); (▼) Puna (Hawaii), (▽) Eleele (Kauai); (■) Kapahi (Kauai); (□) Kula (Maui); (●) Kaanapali (Maui); (○) Kualapuu (Molokai); (∗) Waialua (Oahu); (⊗) Waiahole (Oahu); (+) Kunia (Oahu). Bulk deposition, 0.7095;[29] sea salt aerosol, 0.70917;[30] Hawaiian lava, 0.7035.[31]

show a similar trend, with more depleted $\delta^{34}S$ values of coffee bean measured in altitude coffees (longer distance to the sea). A higher marine influence may be responsible for the differences observed in $\delta^{34}S$ values of coffee beans from different regions, for example, higher values of $\delta^{34}S$ in coffees from Kauai, Maui, and Oahu in comparison to Hawaii, where a greater influence from volcanic activity is expected (Table 1; Figure 3b). Volcanos Kilauea and Mauna Loa located on the island of Hawaii are active, with recent eruptions. Depleted $\delta^{34}S$ values of 0.8−0.9‰ are reported for volcanic sulfur gases (predominantly SO_2) from the Kilauea volcano.[26] This volcanic influence may explain the more depleted $\delta^{34}S$ values of coffee beans produced in the Hawaii region. Nonetheless, monitoring atmospheric, volcanic ash, soil, and precipitation sulfate isotopes will be important to understand how sulfur isotopes of coffee beans reflect these important environmental impacts.

Coffee Bean Strontium Isotopic Composition. The $^{87}Sr/^{86}Sr$ values for the green coffee beans of the different coffee-producing regions are shown in Figure 4. The $^{87}Sr/^{86}Sr$ values varied approximately 0.005. The mean values of $^{87}Sr/^{86}Sr$ of the green coffee beans per region are shown in Table 1, and the values measured for each individual coffee bean sample are shown in Table S3 of the Supporting Information. The highest values were observed in coffees from Kualapuu at Moloaki, Waialua, and Waiahole at Oahu. Lower values of $^{87}Sr/^{86}Sr$ (0.705−0.7052) (Table 1) were measured in coffees from Kau, which may be associated with a greater influence from Kilauea volcano, according to the $^{87}Sr/^{86}Sr$ values reported in literature for Hawaiian lava (see Figure 4). This seems to contribute to the differentiation between these coffees and others (i.e., Molokai and Oahu; Table 1; Figure 3c) derived from regions without active volcanos and at locations correspondent to shorter distances to the sea (where a higher influence from sea salt aerosols on the Sr isotopic composition is expected). Sr isotope ratio analysis has already proved to be an important tool in the assessment of coffee bean geographical origin, whether at regional level[27] or at global level,[3] because it reflects the sources of strontium available during plant growth. In the Hawaiian islands, there are relatively few sources of Sr to the island ecosystems, and these have distinct values that do not vary spatially or temporally and are relatively well-defined[28] (see Figure 4). The sites corresponding to coffee beans with higher $^{87}Sr/^{86}Sr$ values (Kona, Puna, Kaulapuu, Waialua, and Oahu; Figure 4) have in common the proximity of the ocean. In contrast, Kau region coffees, under a closer influence from the Kilauea volcano, showed lower values of $^{87}Sr/^{86}Sr$ (Table 1; Figure 4), approximate to the value reported for Hawaiian lavas.[28] Studies with other Hawaiian plant species have shown that the Sr isotopic signature of plant leaves is a result of the contribution from three main sources of Sr: Hawaiian lavas, mineral aerosol, and seasalt aerosol.[28,29] The same authors state that basalt weathering is still the dominant source of Sr in young ecosystems such as the southernmost part of the Kau region. It is still not possible to evaluate to what extent this is valid for coffee plants, specifically in the case of fruits and seeds, but the results of this work indicate that the main sources of coffee bean Sr should be the Hawaiian lavas as well as the sea salt aerosols.

Coffee Bean Carbon and Nitrogen Isotopic Composition. The C and N isotopic compositions of the green coffee beans samples varied by approximately 8.5 and 5‰, respectively. The mean values of $\delta^{13}C$ and $\delta^{15}N$ of the green coffee beans per region are shown in Table 1 (values measured for each individual coffee bean sample are shown in detail in Table S3 of the Supporting Information). The determination of $\delta^{13}C$ and $\delta^{15}N$ of 30 individual coffee beans from the same coffee sample showed standard deviations of 1.4 and 0.8‰, respectively. In the case of C and N, the results show that these elements did not contribute to the main objective of this work, which was to achieve coffee-producing region differentiation. However, it is important to remember that carbon and nitrogen isotopes are important indicators of ecological change. Further research on these elements' isotopic composition variations during the coffee bean developmental period is necessary to understand how C and N isotopes are integrated in coffee bean tissues.

Combining Isotopic and Multielemental Analysis for Hawaiian Coffee-Producing Region Differentiation. The isotopic compositions of O, S, Sr, C, and N of the Hawaiian coffees did not lead to the differentiation of the five Hawaii coffee-producing regions per se even when all of the results were combined in one multivariate analysis. Nonetheless, the relationships between the isotopic composition of the coffee bean and several environmental factors are important as they may yield understanding of how the coffee bean reflects the plant ecophysiology. It has already been demonstrated that a combination of different elements isotope ratio analysis constitutes a good approach to the differentiation among coffees from different provenances.[3] However, to achieve Hawaiian green coffee bean origin differentiation in this study, it was necessary to combine the results from isotope and multielemental analyses. As previously mentioned, the different coffee bean samples were analyzed for 30 different elements. The results of the multielement analysis are shown in Table 2. Significant differences (ANOVA; $p < 0.05$) were observed among concentrations of the elements Na, Mg, Al, Mn, Ga, Rb, Ba, Pb, Y, La, Ce, Pr, Sm, Nd, Eu, Dy, and Gd for the different coffee-producing regions. Coffees from Molokai showed the most differentiated multielement fingerprint. The concentrations of Al, Fe, Cu, Rb, Sr, and Ce differentiate Molokai from other regions (ANOVA; $p < 0.05$). Similarly, for the

Table 2. 30 Elements Determined (Mean ± SD) in Hawaiian Green Coffees

		mean (ng g^{-1}, ww)														
island where grown		B	Na	Mg	Al	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Rb	Sr	Mo
Hawaii (n = 23)	mean	7009	25276	1904396	1617	10315	26319	83391	106	1241	15099	8412	56	10684	4263	127
	SD	2332	15604	789083	812	29135	11678	39743	61	891	6465	5510	71	5719	2089	204
Kauai (n = 9)	mean	8765	129435	2174746	1156	5236	36259	84690	328	1405	15613	6212	406	6818	6642	99
	SD	2598	62448	880194	595	10294	16817	35199	122	906	2480	1051	221	1342	1512	72
Maui (n = 7)	mean	10008	47223	1641182	4187	2691	47085	95118	252	1027	15375	5875	166	10991	7897	115
	SD	3651	46272	1161311	2121	2488	14509	34817	153	388	3322	1324	54	4083	1519	21
Molokai (n = 2)	mean	14618	<LOD	<LOD	16045	1204	79030	154536	201	1298	21348	7970	87	22844	10438	156
	SD	968	<LOD	<LOD	10302	6	8189	3559	46	83	988	771	30	1729	314	1
Oahu (n = 6)	mean	13276	<LOD	<LOD	3832	1795	83955	116319	228	2749	13541	9019	159	13446	6328	72
	SD	3608	<LOD	<LOD	925	1148	64835	13783	136	1531	1859	4929	106	5371	1867	68

		mean (ng g^{-1}, ww)													
island where grown		Ba	Pb	Bi	Y	La	Ce	Pr	Sm	Nd	Eu	Dy	Th	Sc	Ho	Gd
Hawaii (n = 23)	mean	1191	33	26	<LOD	2	7	<LOD	<LOD	<LOD	40	<LOD	7009	3	4	25276
	SD	1428	66	12	1	7	16	<LOD	<LOD	1	62	<LOD	2332	7	15	15604
Kauai (n = 9)	mean	9355	6	30	19	53	11	<LOD	<LOD	21	371	<LOD	8765	2	<LOD	129435
	SD	5072	16	19	17	32	10	<LOD	<LOD	18	207	<LOD	2598	4	<LOD	62448
Maui (n = 7)	mean	3571	10	22	93	68	26	<LOD	4	53	155	1	10008	7	<LOD	47223
	SD	904	13	1	48	34	14	1	4	26	52	2	3651	4	1	46272
Molokai (n = 2)	mean	2376	14	21	83	72	180	5	6	75	74	1	14618	8	<LOD	<LOD
	SD	843	7	<LOD	46	43	103	7	9	47	27	2	968	2	<LOD	<LOD
Oahu (n = 6)	mean	4203	5	23	60	95	21	2	3	44	180	1	<LOD	3	<LOD	10
	SD	2712	4	5	66	94	18	4	6	46	150	2	<LOD	6	<LOD	11

Kaui region, Ba, Na, and Ga concentrations were significant for the differentiation from other regions. Oahu coffees were different with regard to the Ni concentration, and Hawaii showed similar results except for Nd, La, Y, Co, and Mn (Table 2). Nonetheless, high standard deviations in the different element concentrations per region were observed. Combining all of the results obtained by canonical discriminant analysis allowed the differentiation of the different Hawaiian coffee-producing regions (Figure 5). Three canonical components were obtained with coefficients >0.9 (p < 0.05). It is, however, important to state that the significant group differentiation obtained in this study must be interpreted with caution. As with all multivariate analyses, the ratio of samples to variables should be high. Few samples were available in some of the groups. Because the data analysis was based on more variables than groups in the current data set, new data may not be correctly classified into the groups because of differences not reflected in the current data set. In addition, this study does not include data addressing multiyear variation. The most important next step is to build a model using many more samples including multiple years of harvest. Despite these limitations, our work demonstrates that multielement and isotope analyses can reveal robust patterns of variation among coffees grown in the Hawaiian Islands. Moreover, the characterization of discriminating patterns, elements, and type of analysis in this study presents researchers with information on how the chemistry of the coffee plant responds to specific changes in the environment. Ultimately, this can help illustrate the physiological responses of the coffee plant and seed to agronomic conditions.

In short, it was shown that the combination of S, O, C, N, and Sr isotope analyses with multielement analysis allowed the differentiation of the Hawaiian coffee-producing regions. The results indicate relationships between environmental variables and the green coffee bean isotopic composition. Although additional work is needed to clarify the mechanisms underlying many of these relationships, the results suggest that the isotopic composition of coffees from different regions may to some degree be predictable. If so, this would support the use of stable isotopes as a tool for the verification of coffee origin. In addition, the coffee plant seeds' isotopes may contribute to tracing environmental impacts occurring in Hawaii, in particular if related with volcanic activity, distance to the ocean, and altitude.

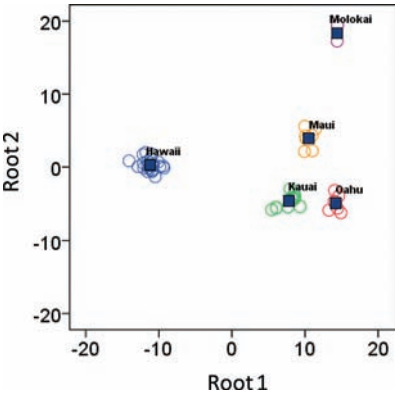

Figure 5. Canonical analysis of isotopic and multielement composition of the 47 green coffee bean samples (squares indicate group centroids).

■ ASSOCIATED CONTENT

Supporting Information. Further details on the operational conditions for the measurement of Sr isotopic and multi element compositions of the green coffee beans are provided in Tables S1 and S2, respectively; experimental results obtained for each individual coffee bean sample included in this study are shown in Table S3; histograms of the results obtained for the determination of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of 30 individual coffee beans of the same sample are shown in Figure S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Krivan, V.; Barth, P.; Morales, A. F. Multielement analysis of green coffee and its possible use for the determination of origin. *Mikrochim. Acta* **1993**, *110*, 217–236.

(2) Serra, F.; Guillou, C. G.; Reniero, F.; Ballarin, L.; Cantagallo, M. I.; Wieser, M.; Iyer, S. S.; Héberger, K.; Vanhaecke, F. Determination of the geographical origin of green coffee by principal component analysis of carbon, nitrogen and boron stable isotope ratios. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 2111–2115.

(3) Rodrigues, C.; Máguas, C.; Prohaska, T. Strontium and oxygen isotope fingerprinting of green coffee beans and its potential to proof authentication of coffee. *Eur. Food Res. Technol.* **2011**, *232*, 361–373.

(4) West, J. B.; Bowen, G. J.; Cerling, T. E.; Ehleringer, J. R. Stable isotopes as one of nature's ecological recorders. *Trends Ecol. Evol.* **2006**, *21*, 408–414.

(5) Farquhar, G. D.; Ehleringer, J. R.; Hubik, K. T. Carbon isotope discrimination and photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1989**, *40*, 503–537.

(6) Scheidegger, Y.; Sauer, M.; Bahn, M.; Siegwolf, R. T. W. Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. *Oecologia* **2000**, *125*, 350–357.

(7) Bowen, G. J.; Wassenar, I. I.; Hobson, K. A. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia* **2005**, *143*, 337–348.

(8) Craig, H. Isotopic variation in meteoric waters. *Science* **1961**, *133*, 1702–1703.

(9) Barbour, M. M. Stable oxygen isotope composition of plant tissues: a review. *Funct. Plant Biol.* **2007**, *34*, 83–94.

(10) Schmidt, H.-L.; Werner, R. A.; Rossman, A. ^{18}O pattern and biosynthesis of natural plant products. *Phytochemistry* **2001**, *58*, 9–32.

(11) Anderson, W. T.; Bernasconi, S. M.; Mckenzie, J. A.; Sauer, M.; Schweingruber, F. Model evaluation for reconstructing the oxygen isotopic composition in precipitation from three ring cellulose over the last century. *Chem. Geol.* **2002**, *182*, 121–137.

(12) Evans, R. D. Physiological mechanisms influencing plant nitrogen isotope composition. *Trends Plant Sci.* **2001**, *6*, 121–126.

(13) Trust, B. A.; Fry, B. Stable sulphur isotopes in plants: a review. *Plant Cell Environ.* **1992**, *15*, 1105–1110.

(14) Wadleigh, M. A. Lichens and atmospheric sulphur: what stable isotopes reveal. *Environ. Pollut.* **2003**, *126*, 345–351.

(15) Google Earth, version 5.0, accessed Nov 2010.

(16) Bowen, G. J. The Online Isotopes in Precipitation Calculator, version 2.2, 2011 (http://www.waterisotopes.org).

(17) Meng, Q.; Liang, T. HNRIS (Hawaii Natural Resource Information System) for Windows, University of Hawaii at Manoa, Honolulu, HI, 2007.

(18) Schultheis, G. *Analysis of Isotope Ratios in Anthropological and Archaeological Samples by High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICP-MS)*; Universität für Bodenkultur am Institut für Chemie (BOKU): Vienna, Austria, 2003.

(19) Swoboda, S.; Brunner, M.; Boulyga, S. F.; Galler, P.; Horacek, M.; Prohaska, T. Identification of Marchfeld asparagus using Sr isotope ratio measurements by MC-ICP-MS. *Anal. Bioanal. Chem.* **2008**, *390*, 487–494.

(20) Barwick, V. J.; Ellison, S. L. R.; Lucking, C. L.; Burn, M. J. Experimental studies of uncertainties associated with chromatographic techniques. *J. Chromatogr., A* **2001**, *918*, 267–276.

(21) Thomsen, V.; Schatzlein, D.; Mercuro, D. Limits of detection in spectroscopy. *Spectroscopy* **2003**, *18*, 112–114.

(22) Barbour, M. M.; Cernusak, L. A.; Farquhar, G. D. Factors affecting the oxygen isotope ratio of plant organic material. In *Physiological Ecology: A Series of Monographs, Texts and Treatises*; Flanagan, L. B., Ehleringer, J. R., Pataki, D. E., Eds.; Elsevier: Oxford, U.K., 2005.

(23) Scholl, M. A.; Ingebritsen, S. E.; Janik, C. J.; Kauahikaua, J. P. Use of precipitation and groundwater isotopes to interpret regional hydrology on a tropical volcanic island: Kilauea volcano area. *Water Resour. Res.* **1996**, *32*, 3525–3537.

(24) Tanz, N.; Schmidt, H.-L. $\delta^{34}S$-value measurements in food origin assignments and sulphur isotope fractionations in plants and animals. *J. Agric. Food Chem.* **2010**, *58*, 3139–3146.

(25) Mizota, C.; Sasaki, A. Sulfur isotope composition of soils and fertilizers: differences between Northern and Southern Hemispheres. *Geoderma* **1996**, *71*, 77–93.

(26) Sakai, H.; Casadevall, T. J.; Moore, J. J. Chemistry and isotope ratios of sulfur in basalts and volcanic gases at Kilauea volcano, Hawaii. *Geochim. Cosmochim. Acta* **1982**, *46*, 729–738.

(27) Techer, I.; Lancelot, J.; Descroix, F.; Guyot, B. About Sr isotopes in coffee 'Bourbon Pointu' of the Réunion Island. *Food Chem.* **2011**, *126*, 718–724.

(28) Chadwick, O. A.; Derry, L. A.; Bern, C. R.; Vitousek, P. M. Changing sources of strontium to soils and ecosystems accross the Hawaiian Islands. *Chem. Geol.* **2009**, *267*, 64–76.

(29) Kennedy, M. J.; Chadwick, O. A.; Vitousek, P. M.; Derry, L. A.; Hendricks, D. M. Replacement of weathering with atmospheric sources of base cations during ecosystem development, Hawaiian Islands. *Geology* **1998**, *26*, 1015–1018.

(30) Capo, R. C.; Stewart, B. W.; Chadwick, O. A. Strontium isotopes as tracers of ecosystem processes: theory and methods. *Geoderma* **1998**, *82*, 197–225.

(31) Whipkey, C. E.; Capo, R. C.; Chadwick, O. A.; Stewart, B. W. The importance of sea spray to the caption budget of a coastal Hawaiian soil: a strontium isotope approach. *Chem. Geol.* **2000**, *168*, 37–48.