Journal of Science and Sustainable Development (JSSD), 2017, 5(1), 1-15

ISSN: 2304-2702 (print); 2414-4479 (online)
DOI: https://doi.org/10.20372/au.jssd.5.1.2017.064

Stable Isotopes and Multi-element Forensics for Tracing the Provenance of Ethiopian Specialty Coffees

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Abstract

Natural diversity in Ethiopian specialty coffees may represent an important opportunity to add value to the economic and social development of the country as well as to the advancement of coffee science. Thus, traceability of geographic origin is primordial. Thus, the objective of this study was to determine stable isotopes and multi-elements fingerprinting of green coffee beans in relation to geographical origins. Green coffee beans obtained from 3 replicated sites per farm in 24 woredas in south-western (SW), west (W), southern (S), north-western (NW) and eastern (E) Ethiopia by hand picking at peak ripening during the 2010-2011 growing season following a multistage purposive random sampling method in a nested design were analysed for %N, $\delta^{15}N$, %C and $\delta^{13}C$, and $\delta^{18}O$ with EA-IRMS; strontium ($^{87}Sr/^{86}Sr$) with MC-ICP-MS and $\delta^{11}B$ with SF-ICP-MS. The results revealed that most stable isotopes were significantly ($P < 0.05$) different across locations. The study further revealed that the concentration of isotopes and multi-elements contributed for canonical discriminating ability of coffee growing regions where the five coffee belts were well grouped. In conclusion, this study revealed that the geographic provenance of specialty coffees produced in Ethiopia could be traced through multi-isotope and multi-element fingerprinting of green coffee beans. Therefore, deliberate or accidental adulteration of lower quality coffees with best quality coffees could be monitored using this method.

Keywords: Coffee origins, coffee isotopes, multi-elements, geographic indications

Introduction

Arabica coffee has evolved in the Ethiopian montane high rainforests with enormous genetic diversity. There are regions producing 'specialty coffees', highly rated on the market that are sometimes mislabelled further along the export-sale chain or mixed with cheaper coffees produced in other regions (Serra *et al*., 2005).Ethiopia has not yet fully exploited its position as the producer of some of the best coffees in the world despite its important role in the 'global coffee value chain' due primarily to the dependence of the sector on the prices set at the world coffee market (Daviron and Ponte, 2005; Petit, 2007). In Ethiopia, the majority of coffee is produced by smallholder farmers without the use of chemicals inputs. Coffees produced in the country have differing taste, aroma, flavour, and texture depending on geographic origin. The region a coffee originates from determines how a coffee tastes due to differences in growing conditions, genetic factors, and processing methods. As a result, the country

possesses distinct specialty coffees such as Harar, Sidamo, Limu, Yirgacheffe, Kaffa, Gimbi (or Nekemte), and Jimma (Ferguson, 2006).

This indigenous diversity in Ethiopian specialty coffees may represent an important opportunity to add value to the economic and social development of the country as well as to the advancement of coffee science. The purpose of checking geographic and/or botanic origin of coffee is, thus, to support the claims and to prevent any adulterations of a product of reputed origin with a cheaper coffee. Hilina (2010) had reported that systematically establishing a relationship between geographic origin of coffee and its quality could lead to the generation of reliable data is a priority area in Ethiopia (Hilina, 2010). To cope with the growing consumption of high quality coffee, improvement and valorisation of coffee quality in the coffee chain provides a new impetus, resulting in the segmentation of the market with substantial premium prices in the world. This scenario has compelled coffee-producing countries to give much attention to genetic, environmental, and management factors to cope with the market demands. Considerable efforts are being made to find out reliable analytical methods for food origin discrimination and authentication. The importance of the coffee market and its globalization caused increased concerns about coffee origins. Consequently, producers have started to offer products with origin labelling. The composition of multi-elements and isotopes of various types in green coffee beans has been widely studied with the aim of indicating the geographic origins of coffee beans (Rodrigues et al., 2011). The studies on composition of multi-elements and isotope ratios, such as carbon, nitrogen, oxygen, boron, and strontium of green coffee beans would indicate how the isotope fractionations and multi-element concentrations undergo during the bean development at different edapho-climatic changes. Thus, traceability of geographic origin is primordial. However, studies of coffee origin discrimination and authentication of specialty coffees has not yet been done in Ethiopia. The existing marketing classifications are subjective, which are based simply on organoleptic assessments and consumer taste. Thus, the objective of this study was to determine stable isotopes and multi-elements fingerprinting of green coffee beans in relation to geographical origins.

Materials and Methods

Site selection, sampling and sample preparation

Twenty-four coffee farms (Figure 1) were selected from the area spanning 3^0 30' to 14^0 55' North, and 33^0 to 48^0 East (Table 2) in Ethiopia. The meteorological information of the areas is presented in Table 1 and Table 2 shows the origin of unwashed coffee

beans in Ethiopia. Ripe coffee cherries were handpicked at their peak ripening phase during 2010/11 crop season. Green coffee beans were subjected to freeze drying just before grinding to fine powder using a hand-held electrical Blade coffee grinder (Bosch MKM 6003 UC, Bean Container Capacity: 75g, Power: 180 Watt). Grinding was assumed to be sufficient when the powder adhered to the ceiling of cap of the grinder, and the powder was immediately packed in plastic cup with a tight stopper, and kept at room temperature until laboratory analysis.

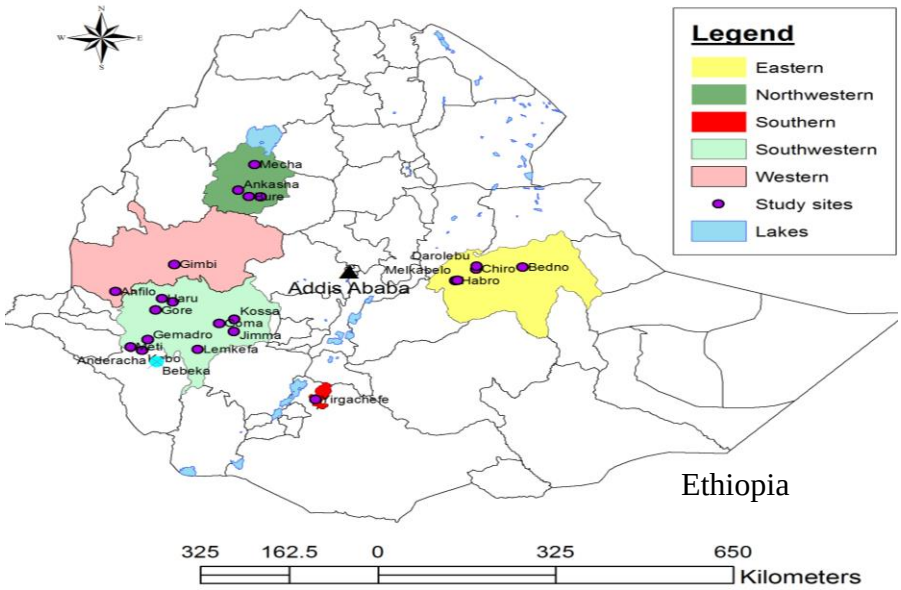

Fig 1. Green coffee bean sample collection sites

Table 1. Mean annual weather data and altitudinal range of the geographical regions (locations)

Geographical Regions (Locations)	Climatic Factors					Altitude range (masl.)
	RF (mm)	Max (^{0}C)	Min (^{0}C)	RH (%)	Sunshine (HRS/day)	
Eastern (26 yrs)	643.7	27.8	12.8	NA	NA	1874 - 2266
North-western (23 yrs)	1140.5	NA	NA	NA	NA	1774 - 2000
Southern (30 yrs)	1345.7	26.6	11.7	69	5.7	2091
South-western (30 yrs)	1564.9	26.1	13.2	73.3	5.4	1150 - 1820
Western (28 yrs)	1385.2	25.6	13.9	70.5	6.5	1800 - 1907

Source: NMSA, Ethiopia (2010); NMSA = National Meteorological Services Agency; NA = data not available; RF = rainfall in mm; Max (^{0}C) = maximum temperature; Min (^{0}C) = Minimum temperature; RH (%) = Relative humidity; HRS = hours; masl. = metres above sea level.

Table 2. **Origins of unwashed green coffee beans in Ethiopia**

Farm	N	Location	Admin. Region	Admin. Zone	Latitude	Longitude
Bebeka	3	Southwest	SNNP	Benchmaji	6.99442	35.5684
Anderacha	3	Southwest	SNNP	Godere	7.23987	35.3169
Kabo	3	Southwest	SNNP	Godere	7.24124	35.3197
Meti	3	Southwest	SNNP	Godere	7.32297	35.1288
Gemadro	3	Southwest	SNNP	Sheka	7.48639	35.4131
Lemkefa	3	Southwest	SNNP	Kafa	7.27274	36.2427
Jimma	3	Southwest	Oromia	Jimma	7.67884	36.8385
Kossa	3	Southwest	Oromia	Jimma	7.95452	36.8468
Goma	3	Southwest	Oromia	Jimma	7.85752	36.5885
Yayo	3	Southwest	Oromia	Illuababora	8.33601	35.8226
Gore	3	Southwest	Oromia	Illuababora	8.14905	35.5369
Gimbi	3	West	Oromia	West Wollega	9.17125	35.8359
Haru	3	West	Oromia	West Wollega	8.40717	35.6396
Anfilo	3	West	Oromia	Kelem Wollega	8.55386	34.8651
Yirgacheffe	3	South	SNNP	Gedeo	6.15848	38.1958
Jabi	3	Northwest	Amhara	West Gojam	10.6918	37.2665
Bure	3	Northwest	Amhara	West Gojam	10.7003	37.0668
Ankasha	3	Northwest	Amhara	West Gojam	10.8436	36.8914
Mecha	3	Northwest	Amhara	West Gojam	11.417	37.1557
Chiro	2	East	Oromia	West Hararghe	9.06989	40.8646
Habro	3	East	Oromia	West Hararghe	8.81697	40.5167
Darolebu	3	East	Oromia	West Hararghe	9.14549	40.8691
Melkabelo	2	East	Oromia	East Hararghe	8.82672	40.5499
Bedeno	3	East	Oromia	East Hararghe	9.11447	41.6335

Laboratory Analysis

Carbon, Nitrogen isotopes and elements measurement

Carbon and Nitrogen Isotopes and %N and %C were measured using a continuous flow (CF) EA-IRMS, Sercon stable isotope mass spectrometers (UK) (OTSUKI, 1983). Finely ground green coffee bean powder (0.95 - 1.4 mg each) rolled in small tin capsules were loaded onto an auto sampler in a duplicate. The samples were purged by a helium (He) flow into a combustion tube and completely oxidized at a temperature of 1000 ^{0}C. A packed GC column removes impurities and separates N_2 and CO_2. A mass spectrometer ionizes gaseous molecules and separates the ions into a spectrum according to their mass-to-charge ratio (m/z), using electric and magnetic fields. The relative abundances of the molecules of different m/z were then found by measuring the currents generated by these separated ion beams. A high vacuum keeps the analyzer pressure low enough (10-5 mbar) to reduce collisions between ions and background gas to an acceptable level. A permanent magnetic field was used (fixed B) and masses were selected by varying the tensions of the electric field V. A universal triple collector was used and B and V were kept constant for each element that has to be measured (Otsuki, 1983).

Oxygen (δ18O) isotope measurement (VSMOW)

It was done via a TC/EA (thermal conversion/elemental analyzer) coupled to an isotope ratio mass spectrometer (IRMS) (20-20, SerCon Ltd, Crewe, UK) (SerCon, 1983.).The green coffee bean powder was further dried overnight at 60-80 °C and stored in desiccators after which samples of a 1 mg each rolled in a silver cup in duplicate were loaded onto an auto sampler. The samples were pyrolyzed at 1400 ^{0}C in a molybdenum lined, aluminium oxide reduction tube filled with glassy carbon and topped with a glassy carbon crucible. The produced N_2 and CO gases were separated via a 1 m gas chromatography (GC) column (E3030, Elemental Microanalysis Ltd., Okehampton, UK) at a temperature of 50 ^{0}C, helium (He) carrier gas pressure of 1.6 bar, Helium (He) flow retention time of about 250 s, and sample analysis time of 1000 s, and analyzed via isotope ratio mass spectrometry (IRMS) for $\delta^{18}O$. The internationally accepted reference values of $\delta^{18}O$-KNO_3 for USGS32 (25.7±0.4 and USGS34 (-27.8±0.4,) (Brand *et al*., 2009) and $\delta^{18}O$-NaNO3 for USGS35 (56.8±0.3 (IAEA, 2004) were used to correct raw $\delta^{18}O$ values to $\delta^{18}O$ (‰) (Otsuki, 1983).

Multi-element and ^{87}Sr isotope analyses

The Multi-elements were analyzed from 43 selected green Arabica coffee samples obtained from 15 coffee growing woredas (districts) in major and potential coffee belts. Five hundred mg green coffee powder was digested with microwave (mls 1200 Mega, Milestone, Shelton, CT, USA) in 6 mL HNO_3 (sub boiled HNO3: 67-69%) + 1 mL H_2O_2 (trace select, > 30%) for 4 minutes (Rodrigues *et al*., 2011).

Strontium isotope ratio measurement

It was determined with MC-ICP-MS instrument (Neptune, ThermoFisher Scientific, Bremen, Germany). From the digestion solution, 2 ml was taken and dried down on hot plate; re-dissolved in 1 mL HNO_3 (sub-boiled) + 1mL Milli Q water added. Matrix separation was accomplished using Sr spec resin (TriSkem international, Lisle, IL, USA): column wash: 2 x 5 mL Milli Q water, 2 mL 0.05 M HNO3, 1 mL 6 M HCl, 5 mL Milli Q water; conditioning of resin: 2 mL 7 M HNO_3 and sample was loaded. Matrix removal was performed with 2 x 2.5 mL 7 M HNO_3 Sr was eluded with 6 mL 0.05 M HNO_3 and the eluded solution was dried down on hot plate and re-dissolved in 3% HNO_3 and the solution was measured. Method parameters maintained during running the instrument were: rf power = 1,300 W, plasma gas flow rate =15 L min-1, aux. gas flow rate = 0.6 - 0.7 L min-1, nebulizer gas flow rate = 0.9 - 1.1 L min-1, resolution = low, and cones = nickel. Cup configuration and isotopes monitored were L4, L3, L2, L1, C, H1, H2 for isotopes 82Kr, 83Kr, 84Sr/84Kr, 85Rb, 85Sr/85Kr, 87Sr/87Rb and 88Sr, respectively. During measurements signal strength of 2V on 88Sr was aimed. Raw data obtained were blank corrected.

Afterwards exponential law (Russell`s law) was applied for internal mass discrimination correction:

$$Rcorr = Robs .m87/(m86)\beta$$

Where; Rcorr is "true" ratio; Robs is measured ratio; m86 and m87 being exact masses of 86Sr and 87Sr isotopes respectively, while ß is mass discrimination factor calculated by taking (88Sr/86Sr) cert = 8.375209 into account:

$$\beta = [\log(88Sr/86Sr)cert/\log(88Sr/86Sr)obs].(\log(m\,88/\,m\,86))-1$$

NIST SRM 987$SrCO_3$ standard solutions was run in a sample-standard bracketing sequence to check stability and accuracy of MC-ICP-MS and for an additional (external) mass discrimination correction using certified value (87Sr/86Sr = 0.71024) (Hammond, 2005; Rodrigues *et al*., 2011).

Multi-element concentration

This was done using ICP-MS instrument (ElementXR, hermoFisher Scientific, Bremen, Germany), MicroMist nebulizer, 200 µL min^{-1}, Glass Expansion, Pocasset, MA, USA & Twister Spray Chamber with Helix, 50 mL cyclonic, borosilicate glass, Glass Expansion, Pocasset, MA, USA (Rodrigues *et al*., 2011). Part of the digestion solution remaining from the strontium isotope determination was transferred in 50 mL volumetric flasks and filled up with Milli Q water after internal standard (In 10 µg L^{-1}) for total quant measurements. External calibration was done based on ICP-MS standards in the range of 0.01 - 300 µg L^{-1} for each element. Determination of limit of detection (LOD) was conducted based on averaged blank raw intensities of all digestion vessels used (n = 20); the standard deviation (sd) was calculated; raw intensities from 10 µg L^{-1} standard was taken into account and LOD was calculated as follows: LOD = 3. sd. 10 ppb/ raw intensities, standard.

The same samples used for ICP-MS analysis were used to measure total quant ICP-OES using ICP-OES instrument from Spectro Analytical Instruments (Arcos, Kleve, Germany) equipped with spectrometer in Paschen-Runge mount for simultaneous monitoring of wavelengths between 130nm and 770 nm. External calibration was done based on ICP-MS standards in the range of 0.01 - 150 mg L-1 for each element. The method parameters maintained were: rf power = 1,400 W, cool gas flow rate =12 L min-1, aux. gas flow rate = 1 L min-1, nebulizer gas flow rate = 0.95 L min-1, wavelengths [nm] =766.491 (K), 280.270 (Mg), 285.213 (Mg), 317.933 (Ca), 422.673 (Ca).

Boron isotope ratio $\delta^{11}B$) measurements

This was carried out by using a single collector double focusing SF-ICP-MS (ELEMENT II, ThermoFisher, Germany). Reagents and standard solutions: Boron standard solutions were prepared from a 10 g L^{-1} commercially available standard (Spex Certi Prep Inc., Metuchen, NJ).

De-ionised water was purified by a Millipore Milli-Q system. The $\delta^{11}B$ values were calculated based on standard reference material NIST 951a Boric acid. All solutions were gravimetrically prepared in polypropylene bottles. For the routine measurement of $\delta^{11}B$ with SF-ICP-MS, the following procedure was used on a set of 43 samples. The instrument was set to maximum sensitivity for boron (NIST SRM 951 solution of 25 $\mu g\ L^{-1}$), by tuning ion lenses and adjusting the nebulizer gas flow rate. Subsequently, in order to reduce the spectroscopic interference of 40Ar4+ on the $^{10}B+$ peak, the auxiliary and nebuliser gas flow were further optimised. Samples were analysed by bracketing with NIST SRM 951 standards; the average $^{11}B/^{10}B$ ratio of NIST SRM 951 measured before and after each sample was used to calculate the $\delta^{11}B$ value of the bracketed sample, which is the recommended routine procedure for $\delta^{11}B$ analysis (Kristof *et al*., 2010).

Optimized instrument settings for measurement of $\delta^{11}B$ with SF-ICP-MS: A quantity of 0.3 - 0.4 mg of the green coffee powder was digested with 7 ml of HNO3 and kept at 105 ^{0}C in the destruction block (Digi PREP Jr Scp Science) for 2 hours. Then the filtrate was injected to SF-ICP-MS. The method parameters maintained according to Kristof *et al*. (2010).

Statistical analysis

The one way ANOVA test and canonical discriminant function analysis were applied to study the significant difference and provenance of isotopes and multi-elements of green coffee beans. Moreover, percentage contribution of predictors to variation of biochemical composition was done using SPSS 16 v2 software.

Results

Isotopes

Significant mean differences were observed between coffees originating from South-western and eastern, western and eastern, and southern and eastern regions (Table 3). However, the difference in %N was non-significant among the regions. With respect to $\delta^{15}N$, significant differences were observed between south-western. Western and southern regions; western and north-western; western and eastern; southern and north-western and southern and eastern regions. Per cent C was non-significant among the locations except between south-western and eastern regions. Locations were found to be non-significant with respect to $\delta^{13}C$, whereas there was highly significant mean difference of $\delta^{18}O$ fractionation between the eastern region and all other regions There was significant mean difference between north-western and both south-western and western regions with respect to $\delta^{18}O$. Furthermore, there was highly significant difference between eastern and all regions; and north-western and southern region with respect to ^{87}Sr isotope composition of green coffee beans. $\delta^{11}B$ was significantly differentiated between southern and

eastern regions and slight difference was also observed between south-western and southern regions.

Table 3. Stable isotopes and percent C and N composition of unwashed green coffee beans (mean± SE)

Location	%N	δ^{15}N (‰)	%C	δ^{13}C (‰)	δ^{18}O (‰)	δ^{87}Sr (‰)	δ^{11}B (‰)
SW(n=33)	2.27±0.04cb	4.8±0.3^{ab}	46.6±0.5^{a}	-27.0±0.2^{a}	31.5+0.4^{c}	0.7063±0^{b}	27.14±1.5^{b}
W(n=9)	2.33±0.02^{cb}	3.4±0.4^{bc}	46.9±0.3^{a}	-26.9±0.6^{a}	31.8±0.5^{c}	0.7068±0.9^{b}	26.98±0.9^{b}
S(n=3)	2.11±0.1^{c}	2.1±0.7^{c}	46.8±0.3^{a}	-26.1±0.2^{a}	33.6±0.3^{bc}	0.7067±0^{b}	33.78±1^{a}
NW(n=12)	2.42±0.1^{ab}	4.9±0.5^{ab}	46.6±1.7^{a}	-27.0±0.4^{a}	34.8±0.7^{b}	0.7069±0^{b}	28.08±2.1^{ab}
E(n=13)	2.62±0.1^{a}	5.6±0.2^{a}	49.5±1.6^{a}	-26.7±0.2^{a}	39.1±0.8^{a}	0.7084±0^{a}	25.6±1.5^{b}
P	0.001	0.002	0.273	0.704	<0.0001	0.006	0.206
SD	0.3	1.8	4.0	1.2	3.7	0.001	5.3
CV (%)	12	37.5	8.6	-4.5	11.1	0.3	19.6

Means followed with the same letter in the column are not significantly different (P<0.05); SW = south-western; W = western; S = southern; NW = north-western; E = eastern; SD = standard deviation; CV = coefficient of variation.

The highest mean values of %N and δ^{15}N were obtained in the eastern region and the least in south with respective standard deviation of 0.3 and 1.8. For %C, the maximum and the minimum mean values were recorded in the eastern, and north-western and south-western regions, respectively (Table 3). With respect to δ^{13}C, although not significant, the maximum and minimum predictors were observed in southern and north-western regions and values deviated from the mean by 1.2. The minimum and maximum respective mean values of δ^{18}O accompanied by standard deviation of 3.7 were obtained from green coffee beans originating from the south-western and eastern regions.

The range for ^{87}Sr isotope was between a minimum mean value in south-western (0.7063±0) and a maximum mean value in the eastern region (0.7084±0). The maximum (33.78±1) mean value for ^{11}B isotope was recorded in the southern, while the minimum (25.6±1.5) was recorded for the eastern region. The study clearly discriminated the eastern coffee growing region from all other regions by its highest mean values for all response variables except for δ^{13}C, which was not sorted out and for ^{11}B, which was sorted out by its lowest mean value. The southern region was also sorted out by its lowest mean values of %N and δ^{15}N and its highest mean values of δ^{13}C and δ^{11}B (Table 3). In this study, the western and southern regions did not vary from each other for all isotopes except for δ^{11}B. Mean values for all isotopes except δ^{13}C were found to be positive, indicating that the samples contained more of the heavy isotopes. On the other hand, δ^{13}C showed negative mean values for all samples, hence,

the dominance of light isotope in the green coffee samples.

The study also showed the existence of a strong correlation between %N and %C (r = 0.859**); %N and $\delta^{18}O$ (r = 0.398**); $\delta^{15}N$ and $\delta^{13}C$ (r=0.405**), and between %C and $\delta^{18}O$ (r=0.281*) with respect to location effect (Table 4).

Table 4. Pearson correlation coefficients of isotopes and elemental compositions of unwashed green coffee beans collected from the five coffee-growing regions in Ethiopia

Factors	$\delta^{15}N$	%C	$\delta^{13}C$	$\delta^{18}O$
%N	-0.019	0.859**	-0.125	0.398**
$\delta^{15}N$		0-.073	0.405**	0.114
%C			-0.018	0.281*
$\delta^{13}C$				0.084

* & ** significant at the P < 0.05 and P < 0.01level, respectively (2-tailed).

Figure 2 shows the percentage contribution for variation in green coffee beans of stable isotopes and elements as affected by location and farm components. The results showed the highest percentage variation was attributed to chance error (78.94%) followed by location (21.06%) and farm (location), while sub-farm, which was used as replication, contribution was negligible with respect to %N. With respect to $\delta^{15}N$ composition of green coffee beans the farm (location) component contributed the highest (69.51%) variation followed by chance error (26.09%), location (4.31%) and sub-farm (0.09%). The highest variation of %C was due to the chance error effect (91.78%), whereas the remaining balance was due to location (3.81%) and sub-farm (4.4) components, while the effect of Farm (Location) was found to be nil. In contrast, the highest variation in $\delta^{13}C$ composition of green coffee beans was observed due to the farm (location) component (72.74%) and chance error effect. The contribution of location and sub-farm was negligible. The composition of $\delta^{18}O$ in green coffee beans was affected more by location (54.85%), farm (location) (25.11%) and chance error components (20.04%) respectively, with sub-farm contribution being negligible.

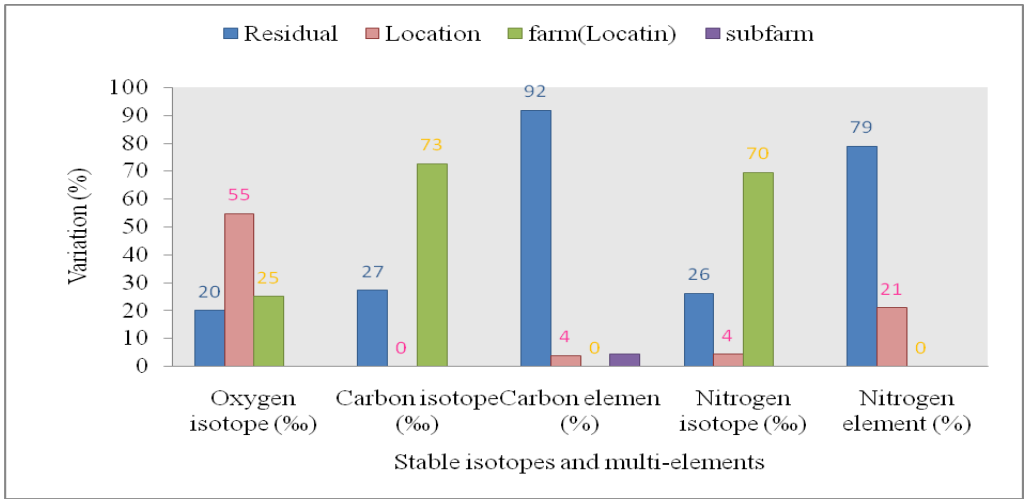

Fig 2. Contribution to the variations (%) in isotope and elemental compositions of green coffee beans as a function of origin

Multi-elements

The Multi-elements (n=21) groups including: Transition-metals (n=8), Alkaline earth metals (n=5), Alkali metals (n=3), Lanthanide series (n=2), Non-metals (n=2), other metals (n=1) were analyzed from 43 green Arabica coffee samples obtained from 15 coffee growing woredas (districts) in major and potential coffee belts. The remaining 19 multi-elements were evaluated for their relative concentration and, hence, contribution to the discriminating ability of coffee origins.

According to a Stepwise Canonical Discrminant Function Analysis, five distinctly isolated centroids were obtained for the geographical locations (Figure 3). Wilks' Lambda test of functions indicated highly significant performances of all functions to process the discriminant factors. The closer the Wilks' lambda value to the zero, the more the variable contributes to the discriminant function. The strength of the functions was assessed based on their Eigen values (13.958, 2.953, 1.845 and 0.843 for function1 through 4, respectively), and revealed that more than 71%, 15%, 9% and 4% of the variance shared the linear combination of variables. The existence of strong association of discriminants with the functions was also indicated by high canonical correlation coefficients (0.966, 0.864, 0.805 and 0.676 for function 1, 2, 3 and 4, respectively).

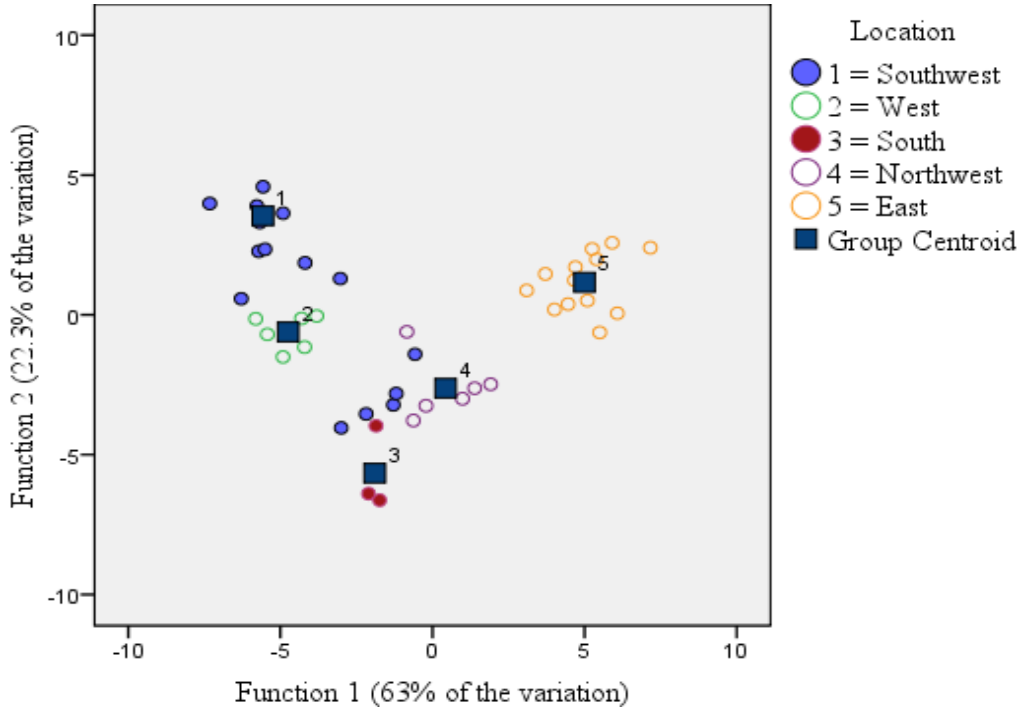

Fig 3.Coffee origins discriminated as a function of multi-elements and isotopes loadings of unwashed coffees

The multi-isotopes and multi-elements combined fingerprinting showed that Ba, followed by $\delta^{18}O$ and Co gave the best discrimination among regions. The elements, including Mo, Ba, Rb, Sr, La, Co, K, 87Sr, Na, B, Ce, Zn, Mg, in descending order of importance had significant contributions to discriminating the Arabica coffee origins in Ethiopia. The elements studied in their groups showed that 100% of the alkali metal and Lanthanide series, 80% of the alkaline earth metals, 50% of the non-metals and 37.5% of the Transition metals studied significantly contributed to the discrimination of the green coffee origins. The study also revealed that isotopes $\delta^{87}Sr$ and $\delta^{11}B$ had relatively less contributions as compared to their counterpart multi-elements. Moreover, the discriminating ability of $\delta^{13}C$ and % C was found to be the poorest in this study.

Discussion

The study revealed that concentrations of the majority of the elements and the stable isotope ratios were significantly different among the coffee growing regions, which could be ascribed to interactions between the coffee plant, local climate, soil type and management. With the exception of $\delta^{13}C$ whose contribution to location was not significant and in consistent with Rodrigues, *et al*., (2011) who reported its negligible contribution to origin discrimination in Hawaii, most isotopes contributed to sort out the farms and locations.

The element composition of the green coffee beans also reflected their relationship with respect to altitude, shade and processing methods of the coffee husbandry practices.

Higher $\delta^{18}O$ values were associated with the coffee beans originating from the eastern region whereas the lowest values were obtained from beans that originated from the south-western region. This result indicates an increasing trend in the content of $\delta^{18}O$ with decreasing atmospheric humidity and increased aridity (low rainfall), which is associated with high evaporative demand for preferential removal of light isotope at low humid or full sun conditions. This result is in agreement with MoA (2003) who reported that the eastern coffee producing region of Ethiopia experiences predominantly low rainfall (500-800 mm per anum), low humidity, and cool temperature as compared to the other coffee producing regions of the country. Todd *et al*., (2002) and USGS (2004) reported that locations with high evaporative demand do discriminate against heavy isotope in preference to light oxygen isotope, which results in higher composition of the residue with heavy $\delta^{18}O$.

Similarly, higher elemental N and $\delta^{15}N$ may be associated with low leaching and low immobilization by soil microbes in the east due to the low annual rainfall and microbial activity compared to other regions. The highest contents of both $\delta^{15}N$ and % N helped to discern the eastern region from the southern region, indicating more intensive care accorded to the coffee plants in the former than in the latter region.

The higher contents of strontium isotope separated from green coffee beans from the eastern region than the strontium isotope separated from green coffee beans of the other regions was probably due to differences in parent material of the soil and exchange reactions. This may be attributed to the predominantly calcium carbonate-rich soils in the region. This suggestion is consistent with the reports of Uloro, (1999) and Zewdie (1999) that most of the parent materials of the soils occurring in the eastern highlands of the country include volcanic rocks such as granites, gneiss and syenites and sedimentary rocks such as lime stones, shale and sandstones, which have ample contents of calcium, magnesium, and sulphur. Consequently, because strontium has an atomic radius similar to that of calcium, it may have readily substituted for Ca in minerals (Todd *et al*., 2002). The highest ^{87}Sr isotopic composition in green coffee beans grown in the eastern region of the country and the least in the southern region of the country could be ascribed to their distinction parental rock and climatic factors.

Isotopic boron ($\delta^{11}B$) also separated all regions except the north-western region. Isotopic fractionation of boron is controlled by the exchange reactions of the boron species

$B(OH)_3$ and $[B(OH)4]^-$, during mineral crystallization, phase-changes in hydrothermal systems, and hydrothermal alteration of rocks. The latter effect results in preferential removal of the $^{10}B(OH)_4$ ion onto clays and results in solutions enriched in $^{11}B(OH)_3$ and, therefore, may be responsible for the large $\delta^{11}B$ enrichment in plants acting as an isotopic signature (Liu, 2014). Analysis of the concentration of multi-elements in green coffee beans enabled canonical discrimination of coffee origins. The elements in minimal concentrations were promising to get sufficient discrimination as compared to those in large concentrations. The results of this study are corroborated by the findings of Rodrigues *et al*. (2011) and Liu *et al*. (2014) who reported that coffee growing regions were discriminated based on multiple isotope and multi-element concentration of green coffee bean obtained from different locations. An inherent limitation of isotope ratio techniques could be due to the mismatch between national borders and climatic borders, delimiting different geographical regions. For this reason, coffees produced in small adjacent countries with similar climatic conditions cannot be distinguished from one another based on different isotope ratio values, while large countries with a large variety of climatic areas may show samples with a range of isotope ratio values, displaying wide dispersion (Serra, *et al*., 2005). It is likely that local growing conditions have the most significant impact on the plants' boron isotope composition (Serra, *et al*., 2005).

Conclusion

The study has demonstrated that coffee beans that originated from the different coffee producing regions in Ethiopia differed significantly in their concentrations of elements and isotopes. It further indicated that multi-year isotope and multi-elements profiling study to a large scale can provide a deeper understanding of what lies behind the differences in the organoleptic qualities of coffee beans originating from the different coffee-growing regions of Ethiopia. This would in turn enable the tapping of existing natural variation in Ethiopian coffee gene pool in the diverse agro-ecology. Therefore, it could be concluded that isotope and multi-element forensics is a promising tool to discern the geographic origins of coffee beans produced in Ethiopia.

Acknowledgements

This paper is part of PhD research work done at Haramaya University. The authors are grateful to the Ministry of Education of Federal Democratic Republic of Ethiopia as well as IUC-JU Soil Fertility Project for funding the research. We are also thankful for the access to the Ghent University, Belgium laboratory providing all facilities required to analyse the coffee bean samples. Prof Dr. Ir. Pascal Boeckx and Professor Jan Diels are thanked for the immense

support in facilitating the laboratories in Ghent, Belgium.

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