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Isotopic signature of the relation between environment and the quality of spatial coffee

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The intrinsic quality of the Bourbon cultivar is well known for a high level of sweetness, intense aroma and pleasant acidity. With the evident relationship between product quality and the environment in mind, the need arises for scientific studies to provide a foundation for discrimination of product origin. Given this context, the aim of this study was to evaluate the use of stable isotopes in discrimination of production environments of Bourbon amarelo coffees from the Serra da Mantiqueira of Minas Gerais by means of statistical modeling. It is believed that upon studying a single variety with high sensory potential, the relation of expression of quality, environment and isotopes may be more evident. Thus, 24 samples of the Bourbon amarelo variety were used for composition of a model through the use of isotopes of $\delta^{18}O$, $\delta^{15}N$, $\delta^{13}C$, %C, %N, δD, and sensory analysis scores. The generated model had a 91.7% accuracy rate for classification of environments, showing in a new way that the use of isotopes may assist understanding of how the Bourbon variety responds to the environmental factors that affect isotope fractionation of C, N, O, and how much the environment collaborates in production of these *terroirs*.

Key words: Isotopic signature, bourbon, quality, geographic origin, coffee.

INTRODUCTION

The quality and complexity of the beverage are the differential that a special coffee can have, making it more valued as it is more rare and exotic. It is grown in various regions of the world. Among these regions with capacity for specialty coffee production, the south of Minas Gerais, Brazil, stands out, and it has been recognized by the Cup of Excellence (COE) as one of the most highly awarded regions in recent years. Part of this success comes from producers in the region returning to planting the Bourbon amarelo cultivar. As one of the purposes in meeting market demand is no longer growing the cultivar for greatest yield but rather the variety which represents quality, the re-emergence of Bourbon amarelo may be observed in production of Brazilian specialty coffees.

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The cultivar manifests early maturity, favoring growing in high altitude locations, leading to the production of chemical compounds that make for sensory quality, resulting in the production of a fine beverage with pleasant nuances for the palate. This notable quality is recognized worldwide and El Salvador is one of the main producer countries. This country is internationally recognized as the great producer of specialty coffee and the Bourbon variety occupies around 70% of the cropped area (Salvadoran Coffee Council, 2009). In this context, designation of origin, which is considered a way of protecting the production location and its products, in addition to adding value, has become a requirement of the international market which has consequently made greater visibility of the product possible. However, by means of measurement of the stable isotope ratios such as $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, information may be obtained about the geographic and botanical origin of many types of food, which makes this one of the most used methodologies in verifying food authenticity and in discrimination of geographic origin (Weckeler et al., 2002). The isotopic composition ($^{18}O/^{16}O$, $^{15}N/^{14}N$, $^{13}C/^{12}C$) of the coffee plant is strongly affected by the environmental conditions of the production location. Rainfall, temperature and relative humidity are characteristic indicating factors of an isotopic signature (Shibuya et al., 2007). In studies on coffee, some authors have shown that the coffee bean has an elemental isotopic composition that varies as a result of the production location, like an isotopic signature or fingerprint (Krivan et al., 1993; Serra et al., 2005; Gonzalvez et al., 2009; Rodrigues et al., 2011). This may be explained by isotope fractionation, which is strongly affected by climate. Thus, fractionation of meteoric water and fractionation of carbon and nitrogen are strongly affected by temperature and by altitude/latitude (Bowen and Revenaugh, 2003), by photosynthetic and respiratory processes, in addition to the strong contribution of gas exchanges in the variation of $^{13}C/^{12}C$ (Ehleringer et al., 2002), and by land use and agricultural practices (Ducatti et al., 2011), respectively. All the studies that cite the use of isotopes as a tool for protected designation of origin for coffees, refer to a continental geographic scale. A recent study performed on the Ilha de Reunião of France (Techer et al., 2011), although on a smaller scale, does not come to work with a regional scale as is proposed in this study. The authors made use of the Sr isotope as a tool of protection of the geographic origin of Bourbon coffees grown in the region of Ilha de Reunião; they related it to the isotopic composition of the rocks, of the meteoric water, of the coffee plants and they compared it in green and roasted coffee beans. Techer et al. (2011) confirmed that the isotopic ratio of $^{87}Sr/^{86}Sr$ found in the rocks and meteoric water are similar to those found in the green and roasted coffee beans, indicating the potential of this tool in geographic discrimination.

However, in light of the aforementioned, it is believed that upon studying a single variety with high potential for quality, the relation of expression of quality, environment and isotopes may be more evident. Thus, the aim of this study was to create a methodology for identifying different production environments of Bourbon amarelo coffees coming from the municipality of Carmo de Minas, a region located in Southeast Brazil.

MATERIALS AND METHODS

Samples and the climate

The coffee beans under study are from the municipality of Carmo de Minas, within the region of the Serra da Mantiqueira of Minas Gerais, which is the second geographic indication for coffee in Brazil. According to the Cup of Excellence, this region has gained worldwide recognition as a producer of high quality coffees (OIC, 2009), which explains the choice of this location to develop this study. The area under study is delimited by the geographic coordinates 22°07’21” Latitude South and 45°07’45” Longitude West (IBGE, 2009). The altitude ranges from a minimum of 864 m to a maximum of 1,634 m. Mean annual temperature is 19.1°C and mean annual rainfall is 1,568 mm (IBGE, 2009).

Design and quality control

Experimental design considered natural processing and hulled/mucilage removed processing, only fruits of the Bourbon amarelo variety, and the three ranges of altitude of below 1,000, 1,000 to 1,200 and above 1,200 m. To ensure the reliability of the samples, each representative lot contained 3 biological replications. All the samples were georeferenced (latitude, longitude and altitude) and collected manually in the ripe cherry stage. They were processed and selected, maintaining the highest quality beans for the purpose of verifying the true effect of the environment.

Quality control of the analyses

Sensory

Sensory analyses were performed only by certified specialty coffee judges, using the methodology proposed by the Specialty Coffee Association of America (SCAA) (LINGLE, 2001). The sensory analysis protocol of the SCAA was used for roasting the coffee, whose coloring must correspond to 58 points on the Agtron scale for the whole bean and 63 points for the ground bean, with tolerance of ±1 point. In each evaluation, five cups of coffee were cupped, representative of the interactions between genotype and environment, performing a session of sensory analysis for each replication, for a total of three repetitions/replications. Each type of processing was evaluated separately. For this study, only the final score of the attributes was considered.

Isotope-ratio mass spectrometry (IRMS)

The green coffee beans were ground in a Retsch mill for 5 min. This was performed three times to achieve a particle size of less than 1 mm. After grinding, the samples were dried in the oven for 12 h at 60°C and placed in tin capsules, folded and weighed again. The weight of the folded capsule was recorded and used to calculate C and N percentage. Elemental analysis was performed in triplicate and the mean and standard deviation was calculated. The certified

Table 1. Summary of the multivariate observations classified according to the linear discriminant model.

Variable		Predicted classification	
		π_1	**π_2**
True Classification	π_1	$n_{(1,1)}$	$n_{(1,2)}$
	π_2	$n_{(2,1)}$	$n_{(2,2)}$
Total	-	N = total number of observations	

reference material (CRM) for validation of the method was Wheat Flour Standard OAS. The values certified for C and N of the CRM were determined with an elemental analyzer calibrated for acetanilide 141 days of the Stable Isotopes and Instrumental Analysis Facility (SIIAF), Lisbon, Portugal.

Combustion mode (EA-C): The stable isotopes of carbon were determined by a Sira II isotope ratio mass spectrometer coupled to a EuroEA elemental analyzer preparation of the sample for combustion-reduction. The nitrogen isotope ratio was determined in an Isoprime (Micromass, Lisbon); the isotope ratio mass spectrometer was coupled to an elemental analyzer EuroEA. The coupling of the elemental analyzers and of the isotope ratio mass spectrometer was through open-split. The isotope proportion of the samples was adjusted according to international standards (IAEA CH6 and IAEA CH7 for carbon isotope ratio and IAEA N1 for nitrogen isotope ratio). The efficiency of the method was verified by means of insertion of laboratory standards among the samples to check stability and to allow correction of “drift” when necessary. Precision was 0.06‰ for determination of the carbon isotope ratio and 0.08‰ for the nitrogen isotope ratio.

Pyrolysis mode (EA-P): The oxygen isotope ratio was determined by an Isoprime, isotope ratio mass spectrometer coupled to a “EuroEA” elemental analyzer for pyrolysis. Pyrolysis occurred at 1,300°C in a glassy carbon reactor with glassy carbon chips and carbon nickel as catalyzers, mounted in a coaxial manner over a ceramic tube. Coupling of the elemental analyzer to the isotope ratio mass spectrometer was through open-split. The isotope ratio of the samples was adjusted by international standards (IAEA 601 and IAEA 602). Analytic performance was verified by means of insertion of laboratory standards among samples to verify stability and to allow correction of deviation when necessary. Precision was 0.14‰.

Data statistics

Models were established by Fisher discriminant analysis, mentioned by Johnson and Wichern (2007) for discrimination of sampled geographic locations. The discriminant function is responsible for explaining the differences among the classification variables (altitude). Classification determines the functions of the variables observed, which allows new objects to be classified in one of the “g” populations. The model created follows the proposed sampling design. The predictive factors tested for the model were: the final sensory analysis score of the coffee samples, delta nitrogen ($\delta^{15}N$), carbon ($\delta^{13}C$), oxygen ($\delta^{18}O$) of the coffee bean, oxygen ($\delta^{18}O$), deuterium (δD), and percentages of carbon (%C) and nitrogen (%N).

Classification of the model

After obtaining the discriminant model and for its validation, a frequency table was obtained, as shown in Table 1, in which each cell represented the total number of observations classified within the following situations: $n_{(1,1)}$ equal to the number of observations belonging to π_1 which were classified in $\pi 1$; $n_{(1,2)}$ equal to the number of observations belonging to π_1 which were classified in $\pi 2$; $n_{(2,1)}$ equal to the number of observations belonging to π_2 which were classified in $\pi 1$ and, finally, $n_{(2,2)}$ represented the number of observations belonging to π_2 which were classified in π_2.

From the results obtained by means of construction of Table 1, it was possible to compute the accuracy rate, which was used to evaluate the quality of classification resulting from Fisher’s discriminant linear function. Thus, this rate was obtained according to expression 1:

$$T = \frac{n_{(1,1)} + n_{(2,2)}}{N} \tag{1}$$

In working with situations that involved more than two classification variables, as is the case of classification by ranges of altitude, a similar procedure was adopted, making due adaptations in discriminant analysis so that the Fisher’s discriminant function and the estimate of the cutoff point were adapted to three classifications.

RESULTS AND DISCUSSION

Classification model-altitude

The model generated by means of the linear response method was able to classify 22 of the 24 samples studied, with an accuracy rate of 91.7%. The relations between the classification variables and the FITS1 are represented in Figure 1.

The relation between the altitude and the $\delta^{15}N$ is represented in Figure 1A. As may be observed, there is a decline in the concentration of the isotopes with increasing altitude. This same observation may be made for $^{18}O/^{16}O$ and for %N (Figure 1C and E, respectively), however, with less evidence of this relation with altitude. Contrary to what was observed for $\delta^{15}N$, $\delta^{18}O$ and %N, the sensory score and the percentage of carbon (Figure 1F and D) manifested a tendency to increase the rates and values as a result of an increase in altitude.

Altitudes below 1000 m had ‰ values for $\delta^{15}N$ (as shown in Table 2) greater than those found at higher altitudes (5.86 ± 3‰). These data show a trend toward lower isotopic abundance of $\delta^{15}N$ in production environments at high altitudes. The same observation

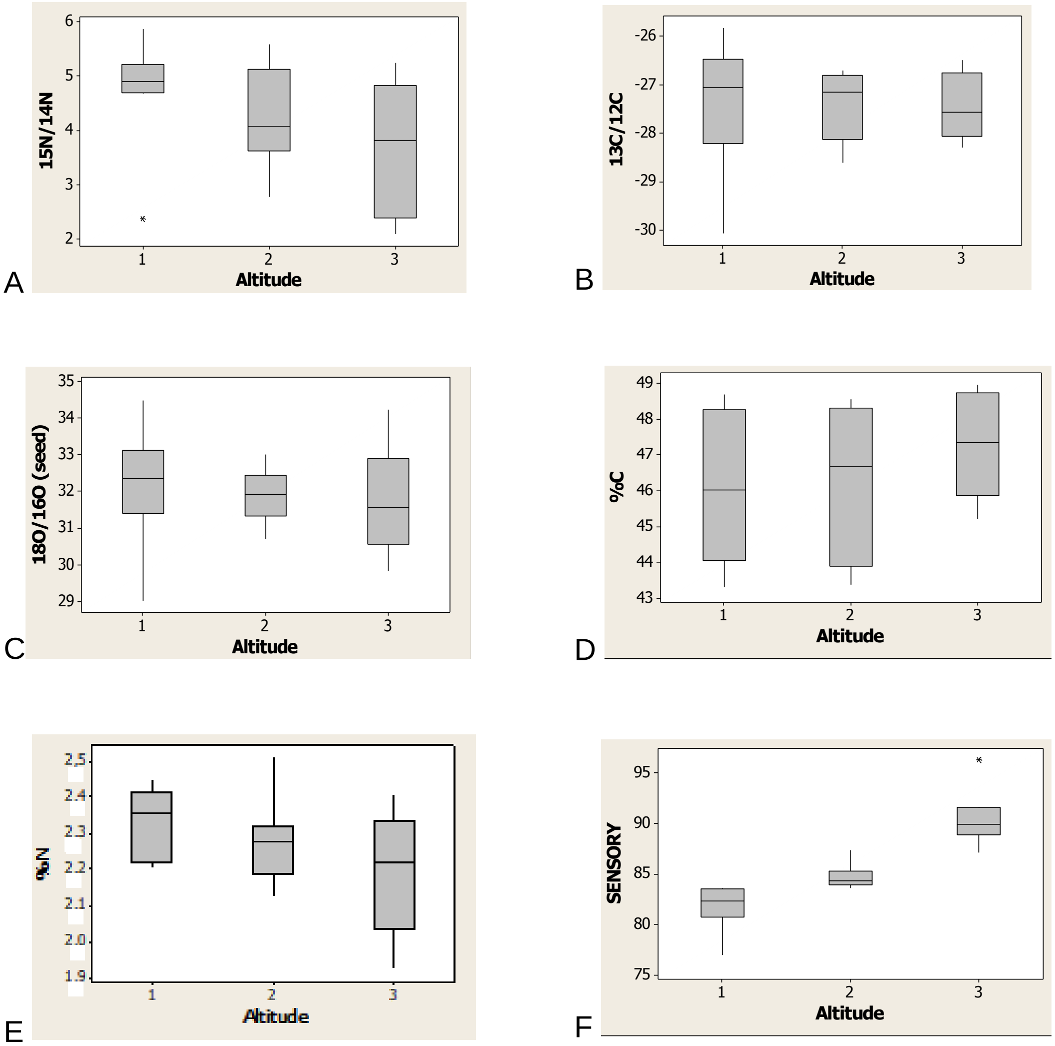


Figure 1. Boxplot of the classification variables (A= $\delta^{15}N$; B=$\delta^{13}C$; C= $\delta^{18}O$; D=%C, E= %N F= final sensory score) of the *Altitude* model. The numbers 1, 2 and 3 correspond to the altitude ranges of below 1000 m, 1000-1200 m and above 1200 m, respectively.

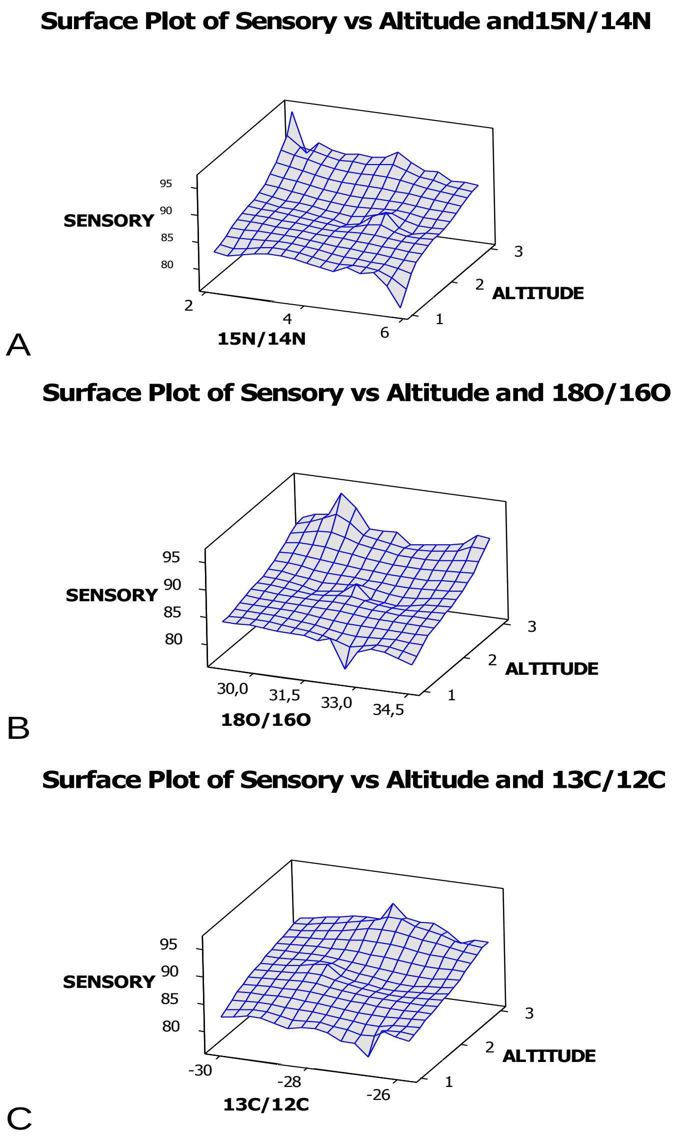
may be made for $^{18}O/^{16}O$ (29.0±34.5‰) in relation to the altitude classifications. A supposition that may explain the result exhibited by $\delta^{15}N$ is isotope fractionation of nitrogen, which is strongly affected by N_2 cycling. Some authors relate this phenomenon to the effects of agricultural practices or even to a result of leaching caused by excess rainfall (Borbemisza, 1982). Nevertheless, greater studies are necessary to determine the cause of this decline in the values found for the coffee beans since there are no reports regarding this fractionation in seeds or in coffee beans. For the same purpose, on a larger geographic scale, some authors studied the composition of a selected element and stable isotope ratio for food products from some European countries (Gonzalvez et al., 2009). Gonzalvez et al. (2009) observed that differences found in $^{15}N/^{14}N$ and $^{13}C/^{12}C$ are related to agricultural practices. Other factors such as rainfall and differences in soil type are reported as having a strong influence on variations of nitrogen cycling patterns and, therefore, on nitrogen isotope composition in plants (Martinelli et al., 1999). As for $\delta^{18}O$, the values found varied for altitude(s) <1000 m (32±34.5‰) and for altitudes >1200 m (29±33‰). However, the differences among the $\delta^{18}O$ values in relation to the altitude classifications are not very evident. It is worth noting that the data presented here are being reported for the first time on a small geographic scale. Therefore, the isotope values found cannot be compared to those found in the literature in reference to a global geographic scale. Nevertheless, it is worth pointing out some results of studies performed in similar geographic areas, such as the study performed by Rodrigues et al. (2011). The authors studied coffees from different islands in Hawaii and were able to discriminate the different

Table 2. Mean values, standard deviation and range of values of C%, N%, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ (seed) and final sensory analysis score of green coffee beans for the three altitude classifications (<1000 m; 1000 to 1200 m; >1200 m).

Altitude	Mean	StanDev	Ranges	Mean	StanDev	Ranges
	$\delta^{18}O$ Natural (seed)			**$\delta^{18}O$ Mucilage removed (seed)**		
1000	33.12	1.19	32.2 - 34.5	31.61	1.63	29.0 - 33.3
1000-1200	31.47	0.65	30.7 - 32.2	32.31	0.57	31.7 - 33
1200	31.54	1.38	29.8 - 33.2	31.94	1.65	30.3 - 34.2
	%C Natural			**%C Mucilage removed**		
1000	45.90	2.48	43.80 - 48.64	46.15	2.11	43.30 - 48.69
1000-1200	45.80	2.22	43.78 - 48.56	46.59	2.26	43.37 - 48.50
1200	47.58	1.5	45.47 - 48.83	47.09	1.53	45.21 - 48.96
	$\delta^{15}N$ Natural			**$\delta^{15}N$ Mucilage removed**		
1000	4.9	0.25	4.68 - 5.18	4.6	1.32	2.38 - 5.86
1000-1200	4.2	0.9	3.58 - 5.59	4.18	1.07	2.78 - 5.25
1200	3.5	1.28	2.3 - 4.99	3.7	1.3	2.09 - 5.24
	%N Natural			**%N Mucilage removed**		
1000	2.27	0.12	2.20 - 2.41	2.37	0.06	2.28 - 2.44
1000-1200	2.26	0.07	2.16 - 2.31	2.29	0.15	2.12 - 2.51
1200	2.18	0.12	2.01 - 2.29	2.19	0.22	1.92 - 2.41
	$\delta^{13}C$ Natural			**$\delta^{13}C$ Mucilage removed**		
1000	-28.63	1.37	-27.31 to -30.05	-26.58	0.52	-25.82 to -27.27
1000-1200	-27.22	0.58	-26.69 to -27.96	-27.61	0.91	-26.82 to -28.60
1200	-27.65	0.72	-26.73 to -28.29	-27.18	0.64	-26.47 to -27.78
	Sensory Natural			**Sensory Mucilage removed**		
1000	81.45	0.97	80.37 - 82.25	81.97	2.82	77 - 83.62
1000-1200	84.37	0.27	84.12 - 84.75	85.09	1.73	83.62 - 87.37
1200	89.78	1.23	88.87 - 91.5	91.25	3.8	87.12 - 96.25

environments by means of the isotopic signature of oxygen $^{18}O/^{16}O$. They observed that the isotope ratio of $\delta^{18}O$ decreases with an increase in altitude; there is a reduction of this element in the chemical composition of the coffee beans. These observations made by the authors validate what was analyzed in this study for the values of $\delta^{18}O$ and $\delta^{15}N$. Nevertheless, it may be seen that in regard to the Bourbon amarelo variety, of high sensory potential, the relationship of quality expression, environment and isotopes is more evident, as shown in Figure 2A, B and C.

A contrary behavior of the $\delta^{15}N$ and $\delta^{18}O$ isotopes may be observed when compared to the sensory score in relation to quality. In other words, in this plot it is possible to visualize that with the increase in altitude classifications there is an increase in the sensory score and a reduction in the respective isotopes. Avelino et al. (2005) performed studies on the quality of Costa Rican coffees and observed a positive relation of the effect of altitude on quality. Although this phenomenon is not very well understood, it was also reported by Barbosa et al. (2012). According to the authors that performed studies on Brazilian coffees, in addition to the quality vs. altitude relation, there is also a relation with latitude as a result of rainfall distribution and temperature. According to Rodrigues et al. (2011), a more refined interpretation of the isotopic abundance of the coffee bean becomes difficult due to the combination of diverse environmental, climatic and physiological processes. In the face of these environmental effects, it has been reported in the literature that isotopic fractionation of oxygen occurs in the plant leaves (Yakir and Sternberg, 2000); however, there are no reports in relation to fractionation in seeds. Taking into consideration that the products formed by photosynthesis are divided up among the locations of greatest demand in the plant (Larcher, 2006) and considering that these products will be deposited and built up during formation and ripening of the coffee fruits,



Surface Plot of Sensory vs Altitude and15N/14N

Surface Plot of Sensory vs Altitude and 18O/16O

Surface Plot of Sensory vs Altitude and 13C/12C

Figure 2. (A) 3D surface plot composed of the variables: stable isotope of nitrogen ($\delta^{15}N$), final sensory score (Sensory) and the altitude classifications (1 = <1000 m, 2 = 1000-1200 m and 3 = >1200 m) represented by the X, Y and Z axes, respectively; (B) 3D surface plot composed of the variables: stable isotope of oxygen ($\delta^{18}O$), final sensory score (Sensory) and the altitude classifications; and (C) 3D surface plot composed of the variables: stable isotope of carbon ($\delta^{13}C$), final sensory score (Sensory) and the altitude classifications.

these events contribute to the distribution of the isotopes studied in the coffee beans. In relation to the isotopes contained in the meteoric water that falls on the soil, authors report that it may reflect the same isotopic abundance found in the rain (Yakir and Sternberg, 2000). Another consideration that may be taken into account is the relation of the water balance in which, by means of transpiration, plants take up the water contained in the soil through the roots and transport it to the transpiration surfaces (Larcher, 2006). Thus, the organic compounds recently synthesized by the plant may contain $\delta^{18}O$, but this may be dependent on fractionation and also on enzymatic, regulatory and synthesis processes. Barbour et al. (2000) report that the isotopic composition of the organic matter of plants is known for reflecting the water absorbed in evapotranspiration conditions at the time the organic matter is formed. From this, it is plausible to infer that the isotopic composition of the $\delta^{18}O$ found in the coffee beans will be reflected in the physiological performance of the coffee plant.

Conclusions

The results presented show that when a single variety of high sensory potential is the point of focus, the relation of quality expression, environment and isotopes is more evident. With the unprecedented nature of the study on such a small geographic scale in mind, greater information in regard to the matter is necessary to clarify such events that create the mystique of the prestigious “terroirs”.

Since the international market requires excellent standards of quality, placing value on products with country of origin labeling, the need is seen for creation and application of methodologies that add value to fine products such as coffees from the Serra da Mantiqueira of Minas Gerais, thus providing for a positive perception of the coffees produced in Brazil.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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