Multielement Analysis of Green Coffee and Its Possible Use for the Determination of Origin

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Abstract. Using instrumental neutron activation analysis (INAA), graphite furnace atomic absorption spectrometry (GFAAS), flame atomic absorption spectrometry (FAAS) and combustion elemental analysis, green coffees of the Arabica species produced in crop year 1987/88 in Colombia, Costa Rica, Cuba, El Salvador, Mexico, Nicaragua, Panamá and Papua New Guinea were analysed for the elements Ba, Br, C, Ca, Co, Cr, Cu, Cs, Fe, H, K, La, Mg, Mn, N, Na, Rb, Sc, Sr and Zn. In accordance with the concentrations determined, the elements could be ranked into five groups: Sc (sub-ppb level); Br, Co, Cr, Cs and La (ppb level); Ba, Cu, Fe, Mn, Na, Rb, Sr and Zn (ppm level); Ca and Mg (‰ level); and C, H, K and N (% level). On the basis of the results obtained, an attempt was made to establish the origin of the green coffee via its elemental composition. Among the investigated elements, manganese was found to be best suited as an indicator for this purpose. However, the elements C, Co, Cs, Na and Rb proved to be of interest too.

Key words: green coffee, elemental composition, instrumental neutron activation analysis, atomic absorption spectrometry, determination of the origin.

The increasing awareness of the important role of trace elements in biological systems has stimulated the extension and refinement of studies in this field which have provided a great deal of information [1–5]. Accordingly, the content of mineral micronutrients and some other trace elements in coffee beans is of great general and special interest, particularly because of the importance of coffee in nutrition and international trade in which it takes second place after petroleum and provides a livelihood for 25 million people around the world [6].

Several investigations have been reported on the determination of elements in green coffee [7–12]. However, most of them have been restricted to small groups of elements, and although a considerable amount of data has been published, it is

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insufficiently consistent. In addition to the general interest in mineral nutrition and in trace elements in coffee derived mainly from its botany and physiology, there is a special aspect worth investigating, i. e., the link between the origin of the coffee and its elemental composition.

The International Coffee Organization (ICO) was created to administer the International Coffee Agreement (ICA) whose objectives are to further international cooperation between coffee exporting and coffee importing countries in order to achieve a reasonable balance between supply and demand [13]. Within the ICO there are currently 51 exporting member countries and the coffees they produce are of different qualities, and in many cases command different prices.

In each coffee-producing country as well as among coffee traders there are tasters who can recognize the country of origin of the coffees they deal with; however, none of them can identify reliably a large number of coffee origins. Moreover the opinions of these tasters are considered subjective, and in cases of arbitration disagreements not infrequently occur between the tasters appointed by the parties involved. In addition, the legal implications when exported or imported coffee is suspected of not being from the origin specified on the contract have motivated coffee producers and traders to find a technique to authenticate the origin of green coffee. However, only a few studies have been conducted on this subject and the majority of them have had little success in achieving their objective.

Dyszel utilized a combination of thermogravimetric analysis and atmospheric pressure chemical ionization mass spectrometry to characterize green coffee [14] but found that the uncertainty of the result made it impossible to identify countries of origin. Following reports of the success of pyrolysis mass spectrometry to characterize orange juices [15] and selected species of bacilli [16], a preliminary study which attempted to characterize ten samples of green coffee (from eight countries of origin) by this method showed that an improvement in reproducibility would be necessary for reliable characterization [17].

A combination of methods used in the characterization of wines [18], involving the investigation of volatile compounds, non-volatile acids, amino acids and some trace elements was also considered for coffee [19]. However, the variability of volatile constituents in coffee caused by the roasting process did not favour such an approach.

The determination of elements, however, could offer a promising alternative because of the well known effect of soil type on the final characteristics of green beans. Inductively coupled plasma atomic emission spectrometry (ICP-AES) was explored for the analysis of green and brewed coffee but, in both cases, reproducibility was poor and the technique abandoned [20, 21].

Neutron activation analysis has proved to be an excellent method for the solution of problems for which high accuracy and precision of analytical results are required [22, 23]. Therefore, in this work, we used instrumental neutron activation analysis (INAA), and as complementary techniques flame and graphite furnace atomic absorption spectrometry (FAAS and GFAAS, respectively) and elemental C-H-N-analysis for a comprehensive major, mineral and trace element analysis of green coffees from 8 different origins. On the basis of the analytical results for 20 elements, the possibility of determining the origin of green coffee by its element characterization was investigated.

Experimental

Gamma-Ray Spectrometer

For counting the gamma-rays of the indicator radionuclides in the INAA, a high resolution gamma-ray spectrometer system consisting of a Ge(Li)-detector with a fwhm of 2.2 keV at 1.332 MeV, a spectroscopy amplifier with gated baseline restorer and pile-up rejector (EG&G ORTEC type 572) and an 8 k multichannel buffer (EG&G ORTEC ADCAM type 918) were used. The gamma-spectra were evaluated using a DEC Professional-350 computer with ORTEC-Geligam V 1.1. software.

Atomic Absorption Spectrometer

For the determination of Cr, Cu, Fe, Mn and Sr by graphite furnace technique and of the elements Ca, Mg and Zn by the flame technique, a Perkin-Elmer type 5000 atomic absorption spectrophotometer with Zeeman background correction combined with a Perkin-Elmer type 3600 data station, a Perkin-Elmer type 400 atomic absorption spectrophotometer with an air/acetylene burner, respectively, were used. For all the elements determined, the spectrometers were equipped with hollow cathode lamps.

Elemental Analyzer

The elements C, H and N were determined by combustion followed by gas chromatographic separation of CO_2, H_2O and N_2 using a Heraeus CHN-o-Rapid elemental analyzer.

Reagents

All reagents used were of pro analysi grade. These as well as the standard stock solutions were supplied by Merck (Darmstadt, Germany). For dilution, doubly-distilled water was used.

Coffee Samples

The description of the investigated samples of green coffee obtained grinded from the Research and Test Unit of ICO (London, Great Britain) are shown in Table 1. The sample materials were coffees of commercial types available on the international markets. In order to determine possible changes of the

Table 1. Investigated samples of green coffee. All samples were of species Arabica and washed on the field

Country of origin	Type	Crop year
Colombia	Medellín Excelso	1987/88
Costa Rica	strictly hard bean	1987/88
Cuba	cristal mountain	1987/88
El Salvador	strictly hard bean	1987/88
México	Altura	1987/88
Nicaragua	A	1987/88
Panamá	Chiriquí	1987/88
Papua New Guinea	X	1981/82
Papua New Guinea	X	1983/84
Papua New Guinea	X	1987/88

contents of the elements in coffee of the same origin within several crop years, Papua coffee of the crop years 1981/82, 1983/84 and 1987/88 was investigated. Before use, the samples were dried over phosphorus pentoxide for 3 days.

Procedures

INAA. For the determination of the elements K, Mn and Na via medium-lived indicator radionuclides, about 70-mg portions of the green coffee samples and the standards were irradiated in polyethylene capsules (Stichting Hart Wool Research Foundation; Amsterdam, The Netherlands). Standards were prepared by pipetting appropriate amounts of the standard solutions into the polyethylene capsules and drying over phosphorus pentoxide in an exsiccator. These capsules (2 samples and 1 standard) were sealed in a polyethylene ampoule and a set of these ampoules was irradiated in the FRM-1 reactor of the TU Munich in Garching (Germany) for 1 hour at a thermal neutron flux of 1.6×10^{13} $cm^{-2} \cdot s^{-1}$.

For the determination of the elements Ba, Br, Ca, Co, Cr, Cs, Fe, La, Rb, Sc and Zn via long-lived indicator radionuclides, sample portions of 150–200 mg and standards were sealed in Suprasil® quartz tubes (Heraeus; Hanau, Germany). The standards were prepared in the same way as described above. The ampoules were irradiated in the GKSS FRG-2 reactor (Geesthacht, Germany) for three days at a thermal neutron flux of 10^{14} $cm^{-2} \cdot s^{-1}$. Before counting, the ampoules were etched with HF (12 mol/l) and HNO_3 (5 mol/l). The basic nuclear data for the indicator radionuclides used are given in Table 2.

AAS. Sample portions of about 200 mg of green coffee were decomposed with a mixture of 3 ml HNO_3 (65 % m/v) and 0.1 ml HF (40 % m/v) in teflon pressure vessels (Berghof; Tübingen, Germany) at 160 °C for 4 hours. After decomposition, the solutions were diluted with water to 25 ml. For the determination of the elements Cr, Cu, Fe, Mn and Sr by GFAAS, a further dilution of the sample solutions with water was necessary. The instrumental parameters used are summarized in Table 3.

Table 2. Main nuclear data for the indicator radionuclides (IRN) used in INAA

Element	IRN	$t_{1/2}$ [d]	γ-rays counted, [keV] (intensity, %)
Ba	^{131}Ba	11.8	496.3 (44)
Br	^{82}Br	1.47	554.3 (71)/776.5 (83)
Ca	^{47}Ca	4.54	1297.1 (75)
Co	^{60}Co	1925	1173.2 (100)/1332.5 (100)
Cr	^{51}Cr	27.7	320.1 (10)
Cs	^{134}Cs	753	604.7 (98)/795.8 (85)
Fe	^{59}Fe	44.5	1099.3 (57)/1291.6 (43)
K	^{42}K	0.52	1524.7 (18)
La	^{140}La	1.68	1596.5 (96)
Mn	^{56}Mn	0.108	846.8 (99)/1811.2 (27)
Na	^{24}Na	0.63	1368.5 (100)/2754.0 (100)
Rb	^{86}Rb	18.6	1076.6 (9)
Sc	^{46}Sc	83.9	889.3 (100)/1120.5 (100)
Zn	^{65}Zn	244.1	1115.5 (51)

Table 3. Instrumental parameters for FAAS (three-slot-burner; air-acetylene flame) and for GFAAS

Parameter	Element							
	Ca[a]	Cr	Cu	Fe	Mg[a]	Mn	Sr	Zn[a]
λ, [nm]	422.7	357.9	324.8	248.3	285.2	279.5	460.7	213.9
Slit, [nm]	2.0	0.7	0.7	0.2	0.7	0.2	0.7	0.7
Lamp[b]	HCL	HCL	HCL	HCL	HCL	HCL	HCL	HCL
Current, [mA]	10	25	15	30	6	20	20	15

[a] FAAS,   [b] HCL = Hollow-cathode-lamp.

Determination of C, H and N. For the determination of C, H and N by combustion elemental analysis, green coffee samples (5–7 mg) were wrapped in tin-foil and combusted in the elemental analyzer. The system was calibrated with acetanilide (Merck; Darmstadt, Germany).

Results and Discussion

General Comments

Coffee, like all other green plants, requires a basic set of major and micro mineral nutrients including the investigated elements N, K, Ca, Mg, Cu, Zn, Fe and Mn [1–3, 5]. Therefore, all plants possess uptake mechanisms capable of moving ions across their cell membranes. In addition, a large number of other elements, ranging from the abundant such as aluminium, sodium and strontium to the rather rare elements like lanthanum and scandium, are accumulated in plants. As far as is known, these elements have no metabolic functions. The results of research into the accumulation of elements in plants show that one of the striking features of ion uptake is the concentration factor, i. e., the ability of plants of the same kind to accumulate ions in concentrations differing considerably from the medium.

Many experiments have been performed on ion uptake but almost all in non-equilibrium conditions in simple solutions, the uptake being measured over a relatively short period. They have shown that there is a characteristic relationship between uptake rate and ion concentration in the solution [24]. However, if more complex solutions were used, many, both inhibitory and stimulatory, interactions were found. Inhibition of the uptake of one ion by another is mostly competitive but non-competitive inhibition has also been observed. It has been found that uptake stimulation is most often associated with a change in Ca^{2+}-concentration, which appears to effect membrane function [24]. In many soils, silicate anions are dominant and these can interact with some cations, and in calcarous soils bicarbonate anions are abundant inhibiting powerfully the uptake of certain cations. Further complications occur because of the fact that many soil solutions contain a wide variety of organic anions derived from the breakdown of lignin, which may strongly affect the uptake of cations [3]. Some additional factors such as environmental pollution and climate may have an influence on the ion uptake.

From the above discussion it is evident that present knowledge of the physiology of ion uptake enables us to assume that the uptake of some ions in coffee beans of

the same sort may be strongly affected by the composition of the soil which varies from region to region. However, due to the complexity of the soil and the multi-factorial nature of the ion uptake process, it is still impossible to predict changes in the accumulation of the elements in coffee beans in accordance with the changes in the composition of the soil. Therefore, a comparison of the content of elements in coffee samples of different origin can be considered a pragmatic approach.

However, in order to determine whether differences in the concentration of a certain element in coffee samples are significant or not, it was necessary to establish the precision of the determination of each element in each sample. For this purpose, replicate determinations were carried out from which the mean values and the standard deviations were calculated. For Cs, Fe, Mn, Na, Rb and Se, five or six replicate determinations were performed by INAA. In the case of Ca, Cr, Fe, Mn and Zn, in addition to INAA, three replicate determinations were carried out using the flame (Ca, Mg, Zn) or graphite furnace (Cr, Fe, Mn) AAS. On the whole, for each sample (apart from some very few cases on which the data for some experimental reason could not be obtained) six results are available for Ca, Cr, Cs, Na, Rb, Sc, and Zn, eight for Mn and nine for Fe. Eight replicate determinations were performed for C, H and N. The results obtained are presented as mean values along with the corresponding standard deviations (when n > 3) in Table 4.

On the basis of the results obtained, the elements investigated can be divided, according to their origin related concentrations in green coffee, into two groups: (1) elements with significantly deviating concentrations, and (2) elements with no significant or no detectable variations.

Manganese

The most interesting results were obtained for the element manganese. After the first results had indicated that this element could be of special interest, it was investigated in more detail by INAA (using samples of about 100 mg) and by GFAAS (using samples of about 200 mg). The results (see Table 4) show that good precision can be obtained by both methods. The relatively low standard deviations also indicate that the investigated materials were of sufficient homogeneity. The agreement between the mean values of both methods can be considered excellent, from which a high achievable degree of accuracy can be concluded.

The concentrations of manganese in the different coffee samples vary between 18 and 58 μg/g. The t-test was applied in order to determine the significance of the difference in manganese content between individual samples, comparing each production region with all the others. The t-test was carried out on two sets of manganese data: (a) the data obtained by INAA (Table 5) and (b) all the data obtained by INAA and GFAAS (Table 6). The regions compared, according to the significance of the difference in the manganese content of the coffee produced in them fall into four groups: (1) those where the difference is highly significant ($\bullet$ > 99.9% statistical reliability), (2) those where it is significant ($\circ$ 99% statistical reliability), (3) those where the difference is probably significant (o 95–99% statistical reliability), and (4) those where no statistical significance can be found for the difference (−, < 95% statistical reliability).

The summaries of the mutual comparison of samples by the t-test given in Tables 5 and 6 show that manganese can be considered a very interesting element for

Table 4. Mean concentrations (µg/g dry weight and, for C, H and N, %-concentrations) and standard deviations for 20 elements in green bean coffee samples from different producing regions determined by different analytical methods. (CEA = combustion elemental analysis)

Mean concentrations and standard deviations

Element	Method	Colombia	Costa Rica	Cuba	El Salvador	México	Nicaragua	Panamá	Papua New Guinea 81/82	Papua New Guinea 83/84	Papua New Guinea 87/88	Literature 6–8
Ba	NAA	5.4 ± 1.4	10.2 ± 2.2	1.9 ± 0.6	2.7 ± 1.2	3.7 ± 0.9	3.0	5.2	2.2 ± 0.5	2.5	3.0 ± 1.4	7–11
Br	NAA	0.4	0.4	1.7	0.3	0.6 ± 0.2	0.3	0.2	0.6	0.3	0.3	0.09–1.2
C	CEA	47.7 ± 0.2	47.2 ± 0.2	47.2 ± 0.1	46.8 ± 0.1	47.0 ± 0.2	47.7 ± 0.2	47.5 ± 0.4	46.3 ± 0.2	46.6 ± 0.22	46.6 ± 0.2	
Ca	NAA	961 ± 260	921 ± 107	1091 ± 86	920 ± 222	1021 ± 196	826 ± 51	1001 ± 114	991 ± 67	877	872 ± 233	
	FAAS	1031 ± 66	938 ± 32	1191 ± 10	1086 ± 45	957 ± 43	911 ± 353	1059 ± 18	1069 ± 37	967 ± 74	1023 ± 10	
	both methods	996 ± 174	930 ± 71	1171 ± 31	1070 ± 53	938 ± 41	869 ± 63	1061 ± 33	1030 ± 64	931 ± 72	948 ± 175	1000–4000
Co	NAA	0.13 ± 0.02	0.15 ± 0.02	0.095 ± 0.020	0.079 ± 0.033	0.18 ± 0.02	0.10 ± 0.01	0.064 ± 0.008	0.055 ± 0.010	0.052	0.055 ± 0.009	0.11–0.35
Cr	NAA	0.88 ± 0.35	0.37 ± 0.09	0.41 ± 0.14	0.50 ± 0.20	0.77 ± 0.67	0.50 ± 0.12	1.00 ± 0.75	0.64 ± 0.17	0.36	0.43 ± 0.23	
	GFAAS	0.72 ± 0.03	0.42 ± 0.21	0.51 ± 0.25	0.67 ± 0.21	0.48 ± 0.04	0.57 ± 0.28	1.01 ± 0.72	0.84 ± 0.18	0.72 ± 0.56	0.73 ± 0.47	
	both methods	0.71 ± 0.06	0.39 ± 0.15	0.46 ± 0.18	0.58 ± 0.21	0.44 ± 0.06	0.53 ± 0.20	1.01 ± 0.66	0.74 ± 0.19	0.38 ± 0.06	0.44 ± 0.16	0.31
Cs	NAA	0.190 ± 0.040	0.050 ± 0.005	0.015 ± 0.002	0.087 ± 0.022	0.128 ± 0.016	0.088 ± 0.009	0.043 ± 0.005	0.109 ± 0.037	0.035 ± 0.003	0.078 ± 0.013	0.025–0.2
Cu	GFAAS	13.7 ± 1.0	15.3 ± 1.1	14.0 ± 0.8	13.2 ± 1.1	12.1 ± 0.9	19.8 ± 0.4	15.0 ± 0.5	12.6 ± 1.1	13.3 ± 1.2	15.1 ± 1.5	1–33
Fe	NAA	41.7 ± 11.5	44.6 ± 2.5	35.8 ± 7.1	44.22 ± 12.3	38.8 ± 11.3	43.2 ± 7.5	40.8 ± 8.3	46.3 ± 10.4	48.0 ± 17.4	39.3 ± 9.2	
	GFAAS	39.4 ± 7.9	36.3 ± 6.0	38.5 ± 7.6	—	41.7 ± 1.8	46.0 ± 11.1	39.0 ± 2.4	58.8 ± 19.3	32.0 ± 11.2	52.8 ± 16.7	
	both methods	40.8 ± 9.8	39.0 ± 7.2	36.9 ± 7.0	—	39.8 ± 9.1	44.3 ± 8.6	40.1 ± 6.4	56.6 ± 20.9	40.0 ± 16.0	44.7 ± 13.8	42–140
H	CEA	7.31 ± 0.14	7.24 ± 0.11	7.06 ± 0.17	6.97 ± 0.13	7.52 ± 0.15	7.17 ± 0.10	7.18 ± 0.15	7.04 ± 0.11	7.04 ± 0.15	7.08 ± 0.09	
K	NAA	14440 ± 228	15604 ± 497	15767 ± 171	15482 ± 466	15012 ± 128	13816 ± 540	15498 ± 647	15250 ± 189	16495 ± 1912	15238 ± 1429	14500–18800
La	NAA	0.0069 ± 0.0011	0.0079 ± 0.0039	0.0059 ± 0.0016	0.0054 ± 0.0014	0.0067 ± 0.0034	0.0035 ± 0.0002	0.0077 ± 0.0006	0.0091 ± 0.0051	0.0150 ± 0.0051	0.0065 ± 0.0031	0.04

Table 4. (Cont.)

Mean concentrations and standard deviations

Element	Method	Colombia	Costa Rica	Cuba	El Salvador	México	Nicaragua	Panamá	Papua New Guinea 81/82	Papua New Guinea 83/84	Papua New Guinea 87/88	Literature 6–8
Mg	FAAS	1814 ± 173	1816 ± 193	1870 ± 92	1794 ± 93	2013 ± 306	1987 ± 152	1891 ± 66	1912 ± 100	1900 ± 98	2017 ± 133	1900–3000
Mn	NAA	45.7 ± 3.2	31.8 ± 2.3	41.9 ± 3.3	22.1 ± 1.1	55.6 ± 4.9	17.7 ± 1.1	21.0 ± 1.6	21.1 ± 1.4	21.7 ± 1.1	23.3 ± 0.7	
	GFAAS	46.5	33.3 ± 6.5	42.4 ± 3.0	22.9 ± 0.6	57.7 ± 4.0	18.0 ± 1.0	22.1 ± 0.8	19.4 ± 1.1	21.1 ± 2.5	20.2 ± 2.0	17–60
	both methods	45.9 ± 2.8	32.4 ± 3.9	42.1 ± 2.9	22.4 ± 1.0	56.4 ± 4.4	17.8 ± 1.0	21.4 ± 1.4	20.5 ± 1.5	21.5 ± 1.6	22.0 ± 2.1	
N	CEA	2.11 ± 0.10	2.17 ± 0.10	2.18 ± 0.05	2.09 ± 0.06	2.22 ± 0.07	2.25 ± 0.07	2.24 ± 0.08	2.00 ± 0.05	1.90 ± 0.09	2.00 ± 0.09	—
Na	NAA	8.7 ± 1.0	8.6 ± 1.2	17.6 ± 1.1	11.5 ± 0.6	7.7 ± 0.8	21.9 ± 0.2	10.0 ± 1.3	6.2 ± 0.9	6.2 ± 0.4	4.8 ± 0.8	4–174
Rb	NAA	40.3 ± 8.3	45.5 ± 5.0	13.5 ± 2.0	38.8 ± 9.1	42.4 ± 6.1	21.2 ± 2.3	27.1 ± 2.6	26.3 ± 3.3	17.4 ± 1.6	26.7 ± 5.8	10–40
Sc	NAA	0.00192 ± 0.00044	0.00061 ± 0.00033	0.00060 ± 0.00010	0.00091 ± 0.00018	0.00053 ± 0.00003	0.00085 ± 0.00015	0.00080 ± 0.00008	0.00134 ± 0.00005	0.00142 ± 0.00014	0.00137 ± 0.00040	0.001–0.004
Sr	GFAAS	6.0 ± 0.6	18.0 ± 0.9	4.9 ± 0.2	4.8 ± 0.1	5.4 ± 0.3	3.2 ± 0.3	16.7 ± 1.2	5.6 ± 0.1	3.8 ± 0.4	4.7 ± 0.4	7.1
Zn	NAA	4.4 ± 0.4	5.5 ± 0.6	5.3 ± 1.0	6.6 ± 1.6	5.1 ± 0.5	5.4 ± 0.5	4.9 ± 0.4	7.7 ± 1.4	5.3	5.3 ± 0.8	
	FAAS	5.6 ± 0.2	6.9 ± 0.7	5.8 ± 0.6	7.7 ± 0.9	6.3 ± 0.1	6.6 ± 1.0	5.2 ± 0.8	7.9 ± 0.9	5.8 ± 1.2	6.5	
	both methods	5.0 ± 0.7	6.2 ± 1.0	5.6 ± 0.8	7.1 ± 1.3	5.7 ± 0.8	6.0 ± 1.0	5.1 ± 0.6	7.8 ± 1.0	5.6 ± 1.0	5.8 ± 0.9	5–10

Table 5. Degree of statistical significance of the difference in the concentration of manganese in coffee samples of different origin obtained by the mutual comparison of the samples using the t-test applied to the results obtained by INAA (n = 5). Statistical significance: ● highly significant, ◉ significant, ○ probably significant, — insignificant

Country of origin	México	Nicaragua	Panamá	Colombia	Costa Rica	Cuba	El Salvador	Papua New Guinea 81/82	Papua New Guinea 83/84	Papua New Guinea 87/88
México										
Nicaragua	●									
Panamá	◉	◉								
Colombia	◉	●	●							
Costa Rica	●	◉	●	●						
Cuba	●	●	●	—	●					
El Salvador	●	◉	—	●	●	●				
Papua New Guinea 81/82	●	◉	—	●	●	●	—			
Papua New Guinea 83/83	●	●	—	●	◉	◉	—	—		
Papua New Guinea 87/88	●	●	○		●	●	—	○	○	

Table 6. Degree of statistical significance of the difference in the concentration of manganese in coffee samples of different origin obtained by the mutual comparison of the samples using the t-test applied to the results obtained by both INAA and GFAAS ($n = 8$). Statistical significance: ● highly significant, ○ probably significant, — insignificant

Country of origin	México	Nicaragua	Panamá	Colombia	Costa Rica	Cuba	El Salvador	Papua New Guinea 81/82	Papua New Guinea 83/84	Papua New Guinea 87/88
México		●	●	●	●	●	●	●	●	●
Nicaragua			●	●	●	●	●	●	●	●
Panamá				●	●	●	—	—	—	—
Colombia					●	○	●	●	●	●
Costa Rica						●	●	●	●	●
Cuba							●	●	●	●
El Salvador								○	—	—
Papua New Guinea 81/82									—	—
Papua New Guinea 83/84										—
Papua New Guinea 87/88										

checking the origin of coffee. As is evident from Table 6, on the basis of 8 determinations (5 replicates by INAA and 3 by GFAAS), the difference in the concentration of manganese is highly significant between Mexico and all other regions, between Nicaragua and all others and between Costa Rica and all others. The coffee from Colombia differs with high significance from all others excluding Cuba, and that from Cuba from all others excluding Colombia; the difference between Colombia and Cuba is only probable. The coffees from Panamá, El Salvador and Papua New Guinea differ highly significantly from coffees of México, Nicaragua, Colombia, Costa Rica and Cuba, but coffees from Panamá, El Salvador and Papua New Guinea do not differ among themselves significantly, and in the most cases do not even differ with probable significance from each other. The high degree of relative constancy of the manganese concentrations in Papua New Guinea coffee over the three crop years 81/82, 83/84 and 87/88 (see also Table 4) is another interesting result suggesting manganese as a possible indicator of the origin of coffee.

On the whole, in 84% of cases the mutal comparison of the coffee samples from different producing regions shows a highly significant difference in the concentration of manganese. The results of the t-test change only insignificantly if it is carried out only on the basis of the replicate determination by INAA without AAS, as can be seen from Tables 5 and 6. By application of the outlier test after Nalimov [25] to the data for the concentrations of Mn, no value was found to be a highly significant or significant outlier.

Possible reasons for the variations in the concentration of manganese in coffees of different origin can be related to varying concentrations of lime [26], phosphorus [27], nitrogen [28], iron [29], organic material [30] and micro organisms [31] in the soil and by the pH- and drainage status of soil [32] as was found in the case of other plants.

Other Elements with Significantly Varying Concentrations (Ba, Co, Cs, Cu, Na, Rb, Sc, Sr)

The alkali metals Cs, Na, and Rb also belong to the elements with significantly varying concentrations in coffee samples of different origin (see Table 4). The average concentrations of Cs vary between 15 and 190 ng/g, those of Na between 5 and 22 μg/g, and the concentrations of Rb between 13 and 46 μg/g. However, the relative constancy of the concentrations of all these three alkali metals in Papua New Guinea coffees of different crop years is not as high as it is in the case of Mn, and the largest deviations were obtained for Cs over the three crop years. Contrary to Cs, Na and Rb, potassium (being the most important nutrient among the alkali metals) shows relatively constant concentrations in all regions. This is a somewhat surprising result as in many higher plants strongly varying potassium concentrations have been observed. For instance, in needles of the first whorl of spruce trees (Picea abies) from different sites in South-West Germany, the concentrations varied between 2,000 and 8,000 μg/g [33].

The t-test was also applied to the results obtained for Cs, Na and Rb in order to allow conclusions regarding the differences in concentration in coffee samples of different origin. As can be seen from Table 7, there is a highly significant difference in the rubidium concentration between México as compared with Nicara-

Table 7. Degree of statistical significance of the difference in the concentration of rubidium in coffee samples of different origin obtained by the mutual comparison of the samples using the t-test applied to the results by INAA ($n = 6$). Statistical significance: ● highly significant, ◐ significant, ○ probably significant, — insignificant

Country of origin	México	Nicaragua	Panamá	Colombia	Costa Rica	Cuba	El Salvador	Papua New Guinea 81/82	Papua New Guinea 83/84	Papua New Guinea 87/88
México										
Nicaragua	●									
Panamá	●	●								
Colombia	—	◐	●							
Costa Rica	—	●	◐	—						
Cuba	●	●	●	●	●					
El Salvador	—	●	●	—	—	●				
Papua New Guinea 81/82	●	○	○	◐	●	●	○			
Papua New Guinea 83/84	●	○	—	●	●	●	○	—		
Papua New Guinea 87/88	●	—	●	◐	●	●	◐	○	○	

gua, Panamá, Cuba and Papua New Guinea. The coffee of Nicaragua differs highly significantly from those of Colombia, Costa Rica, Cuba, El Salvador and México, and significantly from that of Panama; the coffee of Panama differs highly significantly from those of Costa Rica, Cuba, and México, and significantly from those of Colombia and Nicaragua; the coffee of Costa Rica differs highly significantly from those of Cuba, Papua New Guinea, Nicaragua, and Panamá; the coffee of Colombia differs highly significantly from those of Cuba and Nicaragua and significantly from those of Panamá and Papua New Guinea; the coffee of Cuba differs highly significantly from those of El Salvador, México, Nicaragua, Colombia, Costa Rica, Panamá, and Papua New Guinea; the coffee of El Salvador differs highly significantly from those of Nicaragua and Cuba.

Table 8 shows that sodium is a further very interesting element. There is a highly significant difference in sodium concentration between the coffee of Nicaragua and the coffees of all other origins. The same is true for the coffee of Cuba. The coffee of El Salvador differs highly significantly from all other coffees excluding Costa Rica (significant difference) and Panamá (probably significant difference). The coffees of Panamá and Colombia differ highly significantly from several coffees of other origins.

The mutual comparison also shows in many cases highly significant differences in the concentration of Cs (see Table 9). The coffees of Colombia and of Cuba differ highly significantly from all other coffees excluding Papua New Guinea 81/82. Highly significant or significant differences were found for many other combinations.

It is worth mentioning that in those cases where manganese does not differ significantly (Panamá, El Salvador, Papua New Guinea), the alkali metals do. Panamá differs significantly from El Salvador in the concentration of Cs, Panamá from Papua New Guinea in the concentration of Na and Rb, and El Salvador from Papua New Guinea in the concentration of Na and Rb.

As can be seen from Table 4, cobalt is a further minor trace element with essentially varying concentrations (50–180 ng/g). This is well within the usual levels of Co in plants which range from 30 to 1000 ng/g [34]. In some plants, the concentration of Co has been found to vary widely depending on the availability of cobalt in the soil [35].

Although, because results from only three determinations were available for cobalt, the t-test was not applied, the mean values and the corresponding average deviations in Table 4 indicate that cobalt also offers a very interesting possibility for determining the origin of coffee.

The coffee samples from Panamá and Costa Rica contain significantly higher concentrations of Sr (16.7 and 18.0 μg/g, respectively) than coffees of all other origins (3–6 μg/g). This is also true for the concentrations of Sc in the samples from Colombia and Papua New Guinea (1.9 and 1.4 ng/g) as compared with all other samples (0.6–1 ng/g), the concentration of Ba in the sample from Costa Rica (10.2 μg/g) in comparison with all other samples (2.2–5.4 μg/g), and for the concentration of Cu in the sample from Nicaragua (19.8 μg/g) as compared with all other samples (12.1–15.3 μg/g).

The elements discussed above seem able to provide interesting indications regarding the origin of coffee. However, the concentrations of these elements vary

Table 8. Degree of statistical significance of the difference in the concentration of sodium in coffee samples of different origin obtained by the mutual comparison of the samples using the t-test applied to the results obtained by INAA ($n = 5$). Statistical significance: ● highly significant, ◕ significant, ○ probably significant, — insignificant

Country of origin	México	Nicaragua	Panamá	Colombia	Costa Rica	Cuba	El Salvador	Papua New Guinea 81/82	Papua New Guinea 83/84
México									
Nicaragua	●								
Panamá	○	●							
Colombia	—	●	—						
Costa Rica	—	●	—	—					
Cuba	●	●	●	●	●				
El Salvador	●	●	○	●	◕	●			
Papua New Guinea 81/82	○	◕	●	◕	◕	●	●		
Papua New Guinea 83/84	—	●	◕	○	—	●	●	○	
Papua New Guinea 87/88	◕	●	●	●	●	●	●	○	○

Table 9. Degree of statistical significance of the difference in the concentration of cesium in coffee samples of different origin obtained by the mutual comparison of the samples using the t-test applied to the results obtained by INAA (n = 6). Statistical significance: ● highly significant, ◐ significant, ○ probably significant, — insignificant

Country of origin	México	Nicaragua	Panamá	Colombia	Costa Rica	Cuba	El Salvador	Papua New Guinea 81/82	Papua New Guinea 83/84	Papua New Guinea 87/88
México										
Nicaragua	●									
Panamá	●	●								
Colombia	◐	●	●							
Costa Rica	●	●	○	○						
Cuba	●	●	●	●	●					
El Salvador	◐	—	●	●	◐	●				
Papua New Guinea 81/82	—	—	◐	◐	◐	◐	—			
Papua New Guinea 83/84	●	●	—	●	●	●	◐	◐		
Papua New Guinea 87/88	●	—	●	●	●	●	—	—	◐	

in magnitude between 10 ng/g and 10 μg/g. The results for the elements Mn, Na and Rb, with higher concentrations, show lower average deviations and therefore the differences in concentration in samples from different sites can be established more accurately than in the case of Co and Cs which occur in concentrations $< 1 \mu$g/g.

This can in part originate from insufficient homogeneity of biological materials regarding the distribution of trace elements which depends on the concentration level of the given trace element [36]: in general, homogeneity decreases with decreasing concentration level of the elements. The second reason is to be seen in errors in the analytical method. In general, the lower the trace element concentration, the lower the achievable precision and accuracy.

Other Minor and Trace Elements

All the other investigated minor and trace elements do not show large enough variations in concentration in order to be considered as possible indicators of the origin of coffee. Surprisingly constant concentrations throughout the different regions were found for the minor mineral nutrients Ca (930–1,170 μg/g), K (13,800–16,500 μg/g) and Mg (1,800–2,000 μg/g) and the micro mineral nutrients Fe and Zn vary only slightly (36–57 μg/g and 5–8 μg/g, respectively). Bromine, chromium and lanthanum do not show large or clear concentration variations either.

The Major Elements C, H and N

The major elements C and N show very interesting results. In this case, because a small sample of about 5–7 mg was used for analysis, 8 replicate determinations were carried out. The results are given in Table 4. Although only small variations from the mean values occur within the investigated sets of samples, due to high achievable precision the differences in C- and N-concentrations are in many instances highly significant. The results obtained by the t-test for carbon are surveyed in Table 10.

The concentration of carbon varies mainly in connection with certain factors affecting photosynthesis as the main process of dry matter accumulation. Any change in the growth rate is dependent on the rate of net photosynthesis. Several studies have demonstrated the involvement of several mineral nutrients in photosynthesis [37–39], and it has also been possible to observe a reduced rate of photosynthesis under conditions of nitrogen deficiency in various plants including soybeans [40]. Many other factors, including air pollution, were shown to affect photosynthesis. The above factors can explain variations in the concentration of total C.

Highly significant differences also occur in nitrogen (see Table 11), but in a much lower number of mutual combinations of producing regions than in the case of carbon.

The nitrogen cycle is very complex and is dominated by organic processes [41]. Both forms, NH_4^+ and NO_3^- can be taken up by most plants. Significant is NH_4^+ production from organic matter, brought about by various fungi and bacteria. Several other bacteria are involved in the conversion of NH_4^+ to NO_3^-. The varying conditions in one or several stages of the nitrogen cycle enable us to explain the differences in the concentration of nitrogen in samples from different locations.

Table 10. Degree of statistical significance of the difference in the content of carbon in coffee samples of different origin obtained by the mutual comparison of the samples using the t-test applied to the results obtained by combustion elemental analysis (n = 8). Statistical significance: ● highly significant, ◑ significant, ◐ significant, ○ probably significant, — insignificant

Country of origin	México	Nicaragua	Panamá	Colombia	Costa Rica	Cuba	El Salvador	Papua New Guinea 81/82	Papua New Guinea 83/84
México									
Nicaragua	●								
Panamá	◐	—							
Colombia	●	—	—						
Costa Rica	—	●	○	—					
Cuba	○	●	○	●	—				
El Salvador	—	●	●	●	●	●			
Papua New Guinea 81/82	—	●	—	●	●	●	●		
Papua New Guinea 83/84	●	●	—	●	●	●	—	○	
Papua New Guinea 87/88	●	●	—	●	●	●	—	○	—

Table 11. Degree of statistical significance of the difference in the content of nitrogen in coffee samples of different origin obtained by the mutual comparison of the samples using the t-test applied to the results obtained by combustion elemental analysis (n = 8). Statistical significance: ● highly significant, ◑ significant, ○ probably significant, — insignificant

Country of origin	México	Nicaragua	Panamá	Colombia	Costa Rica	Cuba	El Salvador	Papua New Guinea 81/82	Papua New Guinea 83/84	Papua New Guinea 87/88
México		—	—	○	○	—	●	●	●	●
Nicaragua			—	◑	—	○	●	●	●	●
Panamá				○	—	—	●	●	●	●
Colombia					—	—	◑	●	●	—
Costa Rica						—	—	●	●	○
Cuba							◑	—	●	●
El Salvador								◑	●	◑
Papua New Guinea 81/82									○	—
Papua New Guinea 83/84										—
Papua New Guinea 87/88										

Conclusions

From the results of this study, it can be concluded that trace element analysis of green coffee may provide interesting information about its origin. Of the investigated elements Ba, Br, C, Ca, Co, Cr, Cs, Cu, Fe, H, K, La, Mg, Mn, N, Na, Rb, Sc, Sr and Zr, manganese was found to be best suited for this purpose. In 84% of the cases of mutual comparison between eight coffee samples of different origin the difference in the concentration of manganese was statistically highly significant. Other elements of interest for this purpose are Co, Cs, Na, Rb, and the major element C. The main reason for these differences can be seen in the composition of soils.

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