

## Comparative Studies of the Sample Decomposition of Green and Roasted Coffee for Determination of Nutrients and Data Exploratory Analysis

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Os teores de vários nutrientes foram determinados em 35 amostras de cafés verdes e torrados, por espectrometria de absorção atômica em chama (Ca, Mg, Fe, Cu, Mn e Zn), fotometria de emissão atômica em chama (Na e K) e pelo método Kjeldahl (N) após preparação das amostras por dois procedimentos de decomposição via úmida: *i*) sistema aberto (bloco digestor) e *ii*) sistema fechado (forno de microondas convencional com controle de pressão e temperatura). A exatidão dos procedimentos foi verificada analisando-se três materiais de referência certificados do *National Institute of Standards and Technology* (SRM 1573a Folha de Tomateiro; SRM 1547 Folha de Pessegueiro; SRM 1570a Espinafre). A análise dos dados após a aplicação do teste-*t* mostrou que os resultados obtidos no procedimento que empregou decomposição assistida por microondas em frascos fechados foram mais exatos do que os obtidos por bloco digestor com frascos abertos no nível de 95% de confiança. Além da exatidão, outras características favoráveis encontradas foram menores valores de branco analíticos, menor consumo de reagentes e menor tempo de decomposição das amostras. Análises exploratórias dos dados empregando-se Análise por Componentes Principais (PCA) e Análise por Agrupamentos Hierárquicos (HCA) mostraram que os elementos Na, K, Ca, Cu, Mg e Fe foram os principais responsáveis na diferenciação das amostras de cafés verde e torrado.

The contents of some nutrients in 35 Brazilian green and roasted coffee samples were determined by flame atomic absorption spectrometry (Ca, Mg, Fe, Cu, Mn, and Zn), flame atomic emission photometry (Na and K) and Kjeldahl (N) after preparing the samples by wet digestion procedures using *i*) a digester heating block and *ii*) a conventional microwave oven system with pressure and temperature control. The accuracy of the procedures was checked using three standard reference materials (National Institute of Standards and Technology, SRM 1573a Tomato Leaves, SRM 1547 Peach Leaves, SRM 1570a Trace Elements in Spinach). Analysis of data after application of *t*-test showed that results obtained by microwave-assisted digestion were more accurate than those obtained by block digester at 95% confidence level. Additionally to better accuracy, other favorable characteristics found were lower analytical blanks, lower reagent consumption, and shorter digestion time. Exploratory analysis of results using Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) showed that Na, K, Ca, Cu, Mg, and Fe were the principal elements to discriminate between green and roasted coffee samples.

**Keywords:** sample preparation, coffee, nutrients, exploratory analysis

### Introduction

Coffee is an important commodity due to the high consumption of its beverage by people worldwide.<sup>1</sup> Since the value of coffee beans is based on quality, producers and traders have been motivated to make use of extra tools in order to confirm the geographic origin of the coffee.<sup>2,3</sup> In this context, inorganic chemical analyses of

green and roasted coffees may be used to assist the most demanding markets.<sup>1,2,4-8</sup>

Sample preparation still is a critical step in analytical sequence. Among aspects to be taken into consideration in a sample preparation procedure, the most important are the sample size, number and concentration of analytes, type of ashing, compatibility to the measurement equipment, required analytical blanks, time, reagent consumption and suitable precision and accuracy.<sup>9</sup>

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There are several articles in the literature that describe the use of different pre-treatment for coffee solubilization involving wet digestion in a focused microwave system,<sup>1</sup> digester heating block,<sup>2,5,6,8</sup> PTFE (polytetrafluorethylene) pressure vessels,<sup>4</sup> PTFE bombs<sup>8</sup> and dry ashing.<sup>6</sup> Most of these procedures present long waiting-time or use high amount of reagents for sample solubilization. Faster pre-treatment procedures have been proposed by using tetramethylammonium hydroxide (TMAH) in digester heating block (10 min; 80 °C; 500 µL of 25% m/v TMAH solution + 0.35 g soluble coffee)<sup>7</sup> or a focused microwave oven (15 min; 6 mL of concentrated HNO<sub>3</sub> + 0.5 mL H<sub>2</sub>O<sub>2</sub> 30% m/m + 1 g soluble coffee).<sup>8</sup>

Vanadium oxide (V<sub>2</sub>O<sub>5</sub>) was effective as catalyst for wine decomposition in nitric acid for Cd and Pb determination.<sup>10</sup> However, the use of this catalyst for the acid decomposition of green and roasted coffees in digester heating block for macro and micronutrients determination still requires investigation.

This paper reports on the evaluation of wet digestion procedures for the analysis of green and roasted coffees using open vessels in digester heating block and closed vessels heated in a conventional microwave oven system. Calcium, Mg, Fe, Cu, Mn, and Zn were determined by flame atomic absorption spectrometry, Na and K by flame atomic emission photometry, and N by Kjeldahl method. Multivariate exploratory analysis of analytical data using Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) was applied to verify eventual similarity among green and roasted coffee samples.

## Experimental

### *Instruments and apparatus*

A Hitachi Z-8100 Polarized Zeeman Atomic Absorption Spectrometer (Tokio, Japan) was used to determine Ca, Mg, Fe, Cu, Mn, and Zn according instrumental parameters described in Table 1. A Micronal B-262 flame atomic

emission photometer (São Paulo, Brazil) was used to determine Na and K, and a Tecnal TE-036 Kjeldahl apparatus (Piracicaba, Brazil) was used for N determination.

Coffee samples were digested by using a Perkin-Elmer-Anton Paar Multiwave<sup>TM</sup> microwave oven (Graz, Austria) equipped with 50 mL TFM (Teflon Fluor Modified) vessels, and a Tecnal TE-040 digester heating block (Piracicaba, Brazil).

Coffee samples were powdered to particles < 50 µm by employing a Spex 6800 cryogenic mill (Metuchen, USA). A Tecnal TE-648 Willye-type mill (Piracicaba, Brazil) were also used.

### *Reagents, reference solutions and samples*

High purity water (resistivity 18.2 MΩ cm) obtained with a Millipore Rios 5<sup>TM</sup> reverse osmosis and a Millipore Milli-Q<sup>TM</sup> academic deionizer systems (Bedford, MA, USA) were used throughout the study.

All inorganic acids and reagents used in the sample digestion were of analytical grade (HNO<sub>3</sub> 65% v/v – Merck (Darmstadt, Germany), H<sub>2</sub>SO<sub>4</sub> 98% v/v – Mallinckrodt (Phillipsburg, USA), and H<sub>2</sub>O<sub>2</sub> 30% m/m Merck (Darmstadt).

Stock standard solutions (1000 mg L<sup>-1</sup>) of inorganic analytes were prepared by appropriate dilution of respective standard atomic absorption solutions (Normex<sup>TM</sup>, Carlo Erba, Italy). For calibration, analytical solutions within the range of 0.25 - 2.0 mg L<sup>-1</sup> (Ca), 0.050 - 0.80 mg L<sup>-1</sup> (Cu and Zn), 0.50 - 4.0 mg L<sup>-1</sup> (Fe), 0.50 - 8.0 mg L<sup>-1</sup> (K and Na), 0.050 - 0.40 mg L<sup>-1</sup> (Mg), and 0.20 - 2.0 mg L<sup>-1</sup> (Mn) were prepared in 1% v/v HNO<sub>3</sub> by appropriate dilution of the stock solution.

For nitrogen determination by the Kjeldahl method, the combined catalyst was prepared by mixing 1 g Se + 100 g Na<sub>2</sub>SO<sub>4</sub> + 1 g CuSO<sub>4</sub>. A mass of about 10 mg of this mixture was used in each sample digestion vessel. Sodium hydroxide 10 mol L<sup>-1</sup> (Merck, 99% m/m), saturated solution of H<sub>3</sub>BO<sub>3</sub> (Synth, 99.5% m/m), bromocresol green + methyl red indicator, and a standardized 0.1960 mol L<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> solution were also used.

**Table 1.** Instrumental parameters (Hitachi Z-8100 Spectrometer) for Ca, Mg, Cu, Fe, Mn, and Zn determination in green and roasted coffee samples

Instrumental Condition	Ca	Mg	Cu	Fe	Mn	Zn
Radiation source	HCL	HCL	HCL	HCL	HCL	HCL
Lamp current / (mA)	7.5	7.5	7.5	15	7.5	5.0
Wavelength / (nm)	422.7	285.2	324.8	248.3	279.6	213.9
Spectral resolution / (nm)	1.3	1.3	1.3	0.2	0.4	1.3
Linear range / (mg L <sup>-1</sup> )	0.5-2.0	0.05-0.4	0.05-0.8	0.5-4.0	0.2-2.0	0.05-0.8
Instrumental specific parameters						
Background corrector	Zeeman effect					
Measurement mode	Absorbance					
Flame composition	Air/ Acetylene (oxidant)					

A mass of about 10 mg  $V_2O_5$  catalyst was used in the digester heating block procedure. When necessary, samples were filtered (filter paper Framex, medium filtration) and transferred to a 25 mL volumetric flask and the volume made up with deionized water. A volume of 100  $\mu$ L of 5% m/v lanthanum solution (Vetec, 99.99% m/m) was added in each digested sample before Ca and Mg determination to avoid chemical interferences caused mainly by silicates and phosphates.

Five commercial roasted coffee samples were purchased at the local market in Araraquara city (Brazil). Thirty samples of green coffee beans (*Coffea arabica*) were supplied by Brazilian cooperatives located in Minas Gerais and São Paulo States. The green coffee sample identified as PG 8 (Tables 3-6) is an unpulped ripe cherry from Patrocínio city, Minas Gerais State (Brazil).

Standard reference materials produced by National Institute of Standards and Technology (SRM 1573a Tomato Leaves, SRM 1547 Peach Leaves, and SRM 1570a Trace Elements in Spinach) were used to evaluate the accuracy of procedures.

#### Sample preparation procedures and measurements

A mass of green coffee was added to the polycarbonate container until approximately half of its total volume with the magnetic bar, which tightly closed, was adapted to the support and immersed in liquid nitrogen. Samples were pulverized by the impact between coffee beans and the magnetic bar submitted to an oscillating magnetic field of 20 impacts  $s^{-1}$ . The cryogenic mill program consisted of 2 steps: 5 min of pre-cool and 2 min of mill.

One of the selected samples (sample number identification 1) was also ground using a Tecnal TE-648 Willye-type mill (Piracicaba, Brazil) without a previous sample freezing in order to verify eventual contamination by mill components. This sample was identified as number 2. Samples were oven-dried at 60 °C for 12 h.<sup>11-13</sup> The studied sample digestion procedures are described below.

#### Digester heating block

Masses of ca. 400 mg of sample and 10 mg of  $V_2O_5$  catalyst were transferred to glass digester tubes, followed by 3 mL of concentrated  $HNO_3$  plus 2 mL of 30% m/m  $H_2O_2$ . Tubes were heated at 180 °C for 1 h. The final digests were transferred to 25 mL volumetric flasks and the volumes were completed with deionized water. These solutions were directly used for Cu, Fe, Mn, Zn, and Na determination. For Ca, a dilution factor of 10 was necessary. For K and Mg, the solutions were diluted a hundred times. It is a good practice to filter some sample

digests to avoid clogging of the nebulizer of the flame atomic absorption spectrometer.

#### Microwave oven

A mass of ca. 400 mg of sample was transferred to TFM flasks followed by 3 mL of concentrated  $HNO_3$  plus 2 mL of 30% m/m  $H_2O_2$ . It is shown in Table 2 the optimized time-power program of the microwave oven for green and roasted coffee decomposition. The digests were transferred to 25 mL volumetric flasks and the volumes were completed with deionized water. Final solutions were further diluted as described above.

**Table 2.** Optimized time-power program of the microwave oven for green and roasted coffee decomposition

Step	Initial Power/(W)	Final Power/(W)	time/(min)
1	100	800	3
2	800	800	5
3	1000	1000	2
4	0	0	8

#### Kjeldahl method

Masses of ca. 0.15 g of sample and 10 mg of the combined catalyst ( $Se + Na_2SO_4 + CuSO_4$ ) were transferred to glass digester flasks. Volumes of 5 mL of concentrated  $H_2SO_4$  plus 1 mL 30% m/m  $H_2O_2$  were added and the tubes were heated at 350 °C for about 210 min. The resulting solutions were transferred to 25 mL volumetric flasks and the final volumes were completed with deionized water. Ammonia was distilled from a mixture containing 20 mL sample digest plus 20 mL of 10 mol  $L^{-1}$  NaOH solution, and collected in a saturated boric acid solution. This final solution was titrated against standardized sulfuric acid solution.

#### Treatment of data: paired t-test and multivariate analysis

A paired *t*-test was applied to compare the results obtained with digester heating block and microwave oven procedures. It should be commented that the microwave-assisted digestion was elected as standard because it is a well-established tool to provide total digestion without analyte losses.<sup>14</sup>

The PCA and HCA analysis were applied to the data matrix, which consisted in concentration values obtained for the 9 studied analytes in 36 coffee samples. Two algorithms of the Pirouette program (InfoMetrix, Woodinville, WA, USA) version 2.02 were used throughout. The data were auto-scaled (pre-processing) due to the different magnitude orders of the studied variables. The Euclidean distance was used for measuring the similarity and as clustering methods incremental linkage.

## Results and Discussion

The results obtained for 10 samples with the two proposed procedures are depicted in Tables 3 (Na and K), 4 (Ca, Mg and N), 5 (Fe and Mn) and 6 (Zn and Cu). Levels of nutrients obtained for coffee samples were in agreement with those reported in literature.<sup>1,2,4-8</sup> All analyses were performed in triplicates.

Calibration curves with good linear correlation coefficients ( $r > 0.999$ ) were consistently obtained for Ca, Mg, Na, K, Cu, Fe, Mn, and Zn within the working range. The relative standard deviations for the analytes were always  $\leq 2\%$ . The limits of detection (LOD = 3 standard deviation of the blank / slope) in  $\mu\text{g L}^{-1}$  were 29 (Ca), 0.54 (Mg), 1.2 (Na), 1.3 (K), 6.2 (Cu), 11 (Fe), 8.8 (Mn), and 2.0 (Zn).

Analysis of Table 3 suggests contamination by sodium since the samples digested in the open system presented higher levels of Na if compared with closed system. Systematic lower results for Ca (Table 4) were observed, which were attributed to formation of residues in the final digests. For K and Mg (Tables 3 and 4), the application of the paired *t*-test reveals that results obtained by digester block were in agreement with those obtained by microwave oven at 95% confidence level. It is interesting to note that the levels of Na, K and Ca for sample PG 8 (unpulped ripe cherries) are higher than those for pulped ripe cherries, mainly for K.

**Table 4.** Concentrations of Ca, Mg and N in coffee samples

Sample ID <sup>a</sup>	Ca/(mg kg <sup>-1</sup> ) <sup>b</sup>		Mg/(mg kg <sup>-1</sup> ) <sup>c</sup>	
	Block	MW oven	Block	MW oven
PG 5	788 ± 20	1049 ± 3	2054 ± 10	2085 ± 16
PG 6	881 ± 21	1100 ± 80	2008 ± 26	2020 ± 23
PG 7	973 ± 52	1240 ± 20	1868 ± 33	1861 ± 31
PG 8	1870 ± 40	1710 ± 40	1598 ± 11	1609 ± 13
PG 11	885 ± 2	988 ± 11	1778 ± 37	1999 ± 21
R 1	1390 ± 23	1121 ± 12	2190 ± 20	2240 ± 50
R 2	1944 ± 20	1943 ± 31	1830 ± 20	1910 ± 10
R 3	1200 ± 9	1243 ± 18	2280 ± 100	2330 ± 10
R 4	1440 ± 48	1314 ± 54	2280 ± 40	2360 ± 30
R 5	1335 ± 10	1219 ± 19	2170 ± 120	2220 ± 40
Sample ID <sup>a</sup>	N/(%) – Kjeldahl method <sup>d</sup>			
PG 5	2.24 ± 0.04			
PG 6	1.95 ± 0.01			
PG 7	1.82 ± 0.01			
PG 8	1.92 ± 0.02			
PG 11	2.13 ± 0.01			
R 1	2.52 ± 0.04			
R 2	2.14 ± 0.03			
R 3	2.45 ± 0.03			
R 4	2.13 ± 0.04			
R 5	2.34 ± 0.02			

<sup>a</sup>sample identification (PG – Patrocínio/MG green coffee; R – Commercial roasted coffee), <sup>b,c,d</sup>concentration found in literature for Ca (836-1310 mg kg<sup>-1</sup>), Mg (1600-3000 mg kg<sup>-1</sup>) and N (1.90-2.25%), respectively.

**Table 3.** Concentrations of Na and K in coffee samples

Sample ID <sup>a</sup>	Na/(mg kg <sup>-1</sup> ) <sup>b</sup>		K/(%) <sup>c</sup>	
	Block	MW oven	Block	MW oven
PG 5	47.7 ± 1.7	42.3 ± 2.0	1.52 ± 0.04	1.53 ± 0.01
PG 6	67.0 ± 2.8	55.7 ± 3.5	1.52 ± 0.02	1.50 ± 0.06
PG 7	74.7 ± 7.7	73.9 ± 3.5	1.44 ± 0.05	1.48 ± 0.04
PG 8	75.5 ± 2.6	70.7 ± 2.1	2.10 ± 0.04	2.14 ± 0.04
PG 11	59.5 ± 1.9	37.3 ± 0.3	1.84 ± 0.02	1.67 ± 0.01
R 1	96.0 ± 8.5	67.7 ± 3.7	2.13 ± 0.03	1.96 ± 0.05
R 2	90.6 ± 2.8	85.3 ± 1.5	2.79 ± 0.05	2.67 ± 0.02
R 3	111.9 ± 3.5	77.3 ± 1.7	2.09 ± 0.03	2.11 ± 0.04
R 4	84.5 ± 6.0	80.6 ± 7.1	2.27 ± 0.05	2.31 ± 0.07
R 5	100.1 ± 4.2	70.2 ± 4.6	2.05 ± 0.05	2.04 ± 0.02

<sup>a</sup>sample identification (PG – Patrocínio/MG green coffee; R – Commercial roasted coffee); <sup>b,c</sup>concentration values found in literature for Na (10-174 mg kg<sup>-1</sup>) and K (1.38-3.74%), respectively.

According Matos, it is possible to use *in natura* rinds and water from washing coffee process in agricultural areas in order to reduce coffee production costs.<sup>15</sup> Taking into account Brazil production, this could save 430,000 tons of K *per* year, corresponding to around US\$ 120 millions spent with K-based fertilizers for *ca.* 2 millions cultivated hectares. The performance of the decomposition procedure using microwave oven was evaluated after analyzing 3 plant standard reference materials for Na, K, Ca, and Mg determination. For the digester heating block, only N was determined. Results obtained for SRM's (Table 7) were in agreement with certified, non-certified, reference and informative

values at a 95% confidence level. Nevertheless, the found N concentration in all SRM's analyzed was lower than non-certified or reference values. According Fernandes *et al.*<sup>16</sup> in the analyses of vegetable materials for total N determination aiming the determination of crude protein, the Kjeldahl method is the most used. However, the presence of non-protein N (4-9%) in the nitrate form, make this procedure unsuitable, once the quantification of nitrogen in nitrate form is unfeasible due to the incomplete rupture of N-N and N-O chemical bonds during decomposition. The reduction of nitrates to ammonium form can be performed adding salicylic acid with H<sub>2</sub>SO<sub>4</sub> to the samples in the decomposition procedure or by using the Dumas combustion method, which is also employed for total N determination.<sup>17</sup> This method is able to quantify nitrate levels, is cheaper, faster and allows direct combustion of dried samples without the need of using reagents like H<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>O<sub>2</sub>, and catalyst.

The concentrations of micronutrients found in coffee samples are shown in Tables 5 and 6. It can be seen that the digester block is susceptible to zinc contamination and it was ineffective for total Fe total solubilization from roasted coffees. These drawbacks were also observed in rocks and could be circumvented by using either a closed system to sample solubilization or higher amount of reagents and longer time.<sup>18</sup> The Fe content in roasted coffee is typically higher than those found in green coffees (Table 5). It is interesting to note that the concentration value obtained for Fe in

**Table 5.** Concentrations of Fe and Mn in coffee samples

Sample ID <sup>a</sup>	Fe/(mg kg <sup>-1</sup> ) <sup>b</sup>		Mn/(mg kg <sup>-1</sup> ) <sup>c</sup>	
	Block	MW oven	Block	MW oven
PG 5	32.4 ± 0.7	37.0 ± 0.8	31.2 ± 0.1	31.0 ± 0.4
PG 6	41.4 ± 1.0	42.2 ± 1.1	14.4 ± 0.2	14.7 ± 0.1
PG 7	97.9 ± 5.2	107.5 ± 4.7	16.2 ± 0.2	13.4 ± 0.3
PG 8	78.7 ± 4.8	107.0 ± 3.4	23.5 ± 1.0	23.6 ± 0.1
PG 11	25.8 ± 0.5	26.8 ± 0.7	24.1 ± 0.2	24.0 ± 0.4
R 1	51.4 ± 2.1	61.0 ± 0.2	19.8 ± 0.9	22.0 ± 1.0
R 2	192.0 ± 5.2	282.3 ± 15.2	26.4 ± 0.4	27.4 ± 0.1
R 3	51.7 ± 1.7	69.5 ± 1.6	26.2 ± 1.3	27.9 ± 0.1
R 4	154.2 ± 5.6	233.7 ± 2.2	31.7 ± 1.6	36.0 ± 0.6
R 5	66.7 ± 1.8	85.0 ± 8.5	23.9 ± 0.2	24.5 ± 0.5

<sup>a</sup>sample identification (PG – Patrocínio/MG green coffee; R – Commercial roasted coffee), <sup>b,c</sup>concentration values found in literature for Fe (25.0-140 mg kg<sup>-1</sup>) and Mn (14.0-60.0 mg kg<sup>-1</sup>), respectively.

**Table 6.** Concentrations of Zn and Cu in coffee samples

Sample ID <sup>a</sup>	Zn / (mg kg <sup>-1</sup> ) <sup>b</sup>		Cu / (mg kg <sup>-1</sup> ) <sup>c</sup>	
	Block	MW oven	Block	MW oven
PG 5	9.34 ± 0.10	6.17 ± 0.07	14.9 ± 0.3	14.3 ± 0.3
PG 6	16.8 ± 0.2	6.73 ± 0.90	14.0 ± 0.2	14.5 ± 0.3
PG 7	9.67 ± 0.24	7.81 ± 0.37	14.0 ± 0.7	14.3 ± 0.6
PG 8	13.6 ± 0.6	5.89 ± 0.41	16.0 ± 0.4	16.7 ± 0.4
PG 11	4.55 ± 0.03	4.24 ± 0.06	12.7 ± 0.2	13.1 ± 0.1
R 1	10.8 ± 0.2	7.14 ± 0.33	13.3 ± 0.2	12.5 ± 0.5
R 2	12.8 ± 0.5	7.08 ± 0.34	18.8 ± 0.3	18.7 ± 0.3
R 3	5.23 ± 0.44	4.65 ± 0.42	14.5 ± 0.3	14.8 ± 0.4
R 4	15.5 ± 0.3	6.17 ± 0.02	18.1 ± 0.2	17.1 ± 0.3
R 5	4.80 ± 0.32	5.31 ± 0.37	14.7 ± 0.2	13.7 ± 0.3

<sup>a</sup>sample identification (PG – Patrocínio/MG green coffee; R – Commercial roasted coffee), <sup>b,c</sup>concentration values found in literature for Zn (3.00-10.0 mg kg<sup>-1</sup>) and Cu (1.00-33.0 mg kg<sup>-1</sup>), respectively.

**Table 7.** Concentration values determined for Na, K, N, Ca, Mg, Fe, Mn, Zn and Cu in three plant standard reference materials

Elements	SRM	SRM 1573 Tomato leaves	SRM 1547 Peach leaves	SRM 1570 Spinach
Na/(mg kg <sup>-1</sup> )		136 ± 4 (C) 140.8 ± 0.2 (O)	24 ± 2 (C) 25.8 ± 0.3 (O)	18180 ± 43 (C) 17709 ± 185 (O)
K/(%)		2.70 ± 0.05 (C) 2.67 ± 0.02 (O)	2.43 ± 0.03 (C) 2.30 ± 0.05 (O)	2.90 ± 0.05 (C) 2.72 ± 0.06 (O)
N/(%)		2.92 (NC) 2.73 ± 0.003 (O)	2.96 (NC) 2.55 ± 0.004 (O)	5.68 ± 0.13 (RV) 5.19 ± 0.01 (O)
Ca/(%)		5.05 ± 0.09 (NC) 5.06 ± 0.13 (O)	1.56 ± 0.02 (C) 1.57 ± 0.01 (O)	1.527 ± 0.041 (C) 1.440 ± 0.013 (O)
Mg/(%)		1.2 (NC) 1.20 ± 0.015 (O)	0.432 ± 0.008 (C) 0.433 ± 0.003 (O)	0.89 (IV) 0.90 ± 0.011 (O)
Fe/(mg kg <sup>-1</sup> )		368 ± 7 (C) 357 ± 2 (O)	218 ± 14 (C) 195.9 ± 9.8 (O)	(NS) 235.5 ± 1.7 (O)
Mn/(mg kg <sup>-1</sup> )		246 ± 8 (C) 247 ± 3 (O)	98 ± 3 (C) 88.6 ± 0.3 (O)	75.9 ± 1.9 (C) 71.6 ± 0.3 (O)
Zn/(mg kg <sup>-1</sup> )		30.9 ± 0.7 (C) 30.3 ± 0.2 (O)	17.9 ± 0.4 (C) 16.6 ± 0.1 (O)	82 ± 3 (C) 76 ± 1 (O)
Cu/(mg kg <sup>-1</sup> )		4.70 ± 0.14 (C) 4.45 ± 0.14 (O)	3.7 ± 0.4 (C) 3.9 ± 0.2 (O)	12.2 ± 0.6 (C) 12.2 ± 0.2 (O)

(C) – certified concentration value; (O) – obtained concentration value; (NC) – non-certified concentration value; (RV) – reference concentration value; (IV) – information concentration value; (NS) – not specified concentration value.

sample PG 8 (unpulpd ripe cherries) is higher than those for pulped ripe cherries (green coffee samples identified as PG 5, PG 6, and PG 11).

The difference between the concentrations of Fe obtained for a green coffee ground by cryogenic mill (sample PG 6) and Willye-type steel mill (sample PG 7) can be attributed to abrasion of inner parts of the Willye-type mill. Contamination by Fe and Cr was also observed for plant material ground in steel mill due to the releasing of these elements to the samples by the overheating of the mill.<sup>19</sup> The obtained values for Cu, Fe, Mn and Zn in three SRM's were in agreement with certified values at 95% confidence level (see Table 7).

The most important commercial species of coffee are *Coffea arabica* and *Coffea canephora*, usually known as *arabica* and *robusta* varieties, respectively. *Coffea robusta* is more used in production of soluble coffee, and the solids extraction rate in fabrication process of this coffee is higher than when using *Arabica*.<sup>20</sup> This can explain differences for some macro and micronutrients levels found in green (*arabica*) and roasted coffee samples (probably with the addition of *robusta*), in addition to factors involved in the industrial process of roasted coffee.<sup>1</sup>

The PCA and HCA analysis were applied after auto-scaling data due to the different magnitude orders of the concentrations.<sup>21,22</sup> The PCA showed that with only six principal components it is possible to describe 91.4% of data, being 32.3% of the total variance described by the first principal component (PC1), 18.6% by PC2, 14.8% by PC3, 10.7% by PC4, 10.0% by PC5 and 5.0% by PC6. The simultaneous analysis of the scores and loadings graphics (Figures 1a and 1b) show that PC1 separates the

samples in relation to Ca, K, and Na concentrations, while PC3 separates the samples in relation to Cu, Fe and Mg concentrations. The commercial roasted coffee samples (samples R1-R5) presented higher concentrations of Mg (samples R1, R3, and R5), Na, Ca, and K (samples R2 and R4). It should be commented that samples R2 and R4

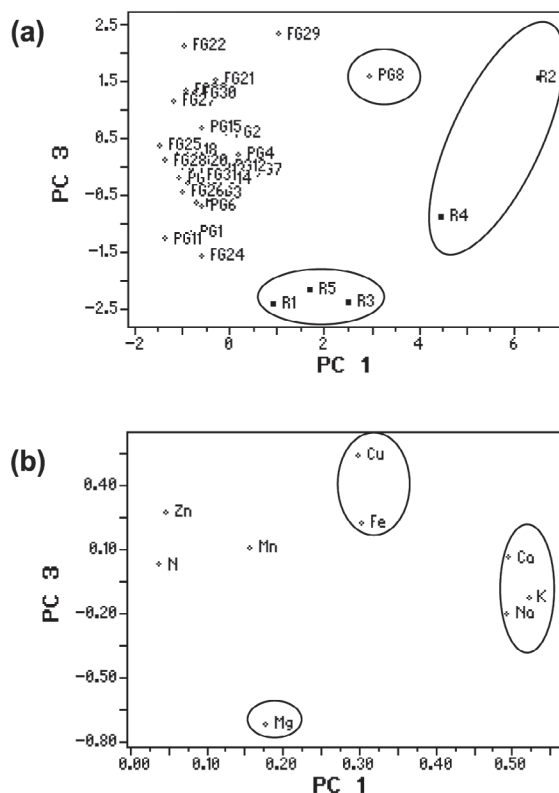


Figure 1. Principal component analysis (PC 1  $\times$  PC 3) showing Scores (a) and Loadings (b) graphics.

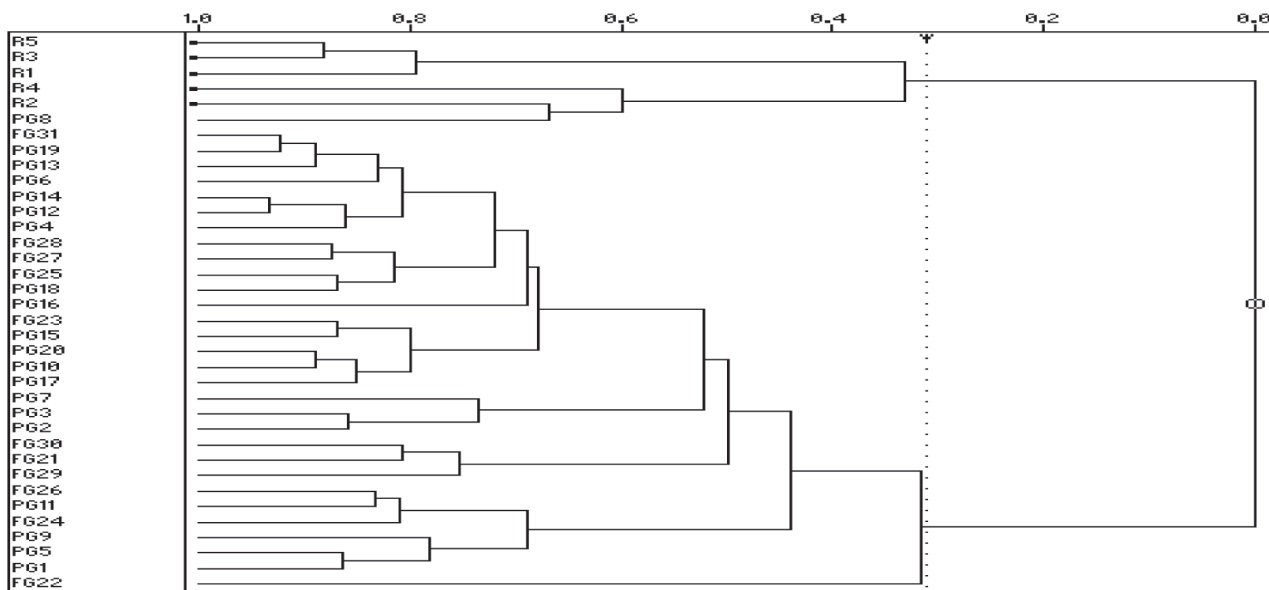


Figure 2. Hierarchical cluster analysis. Similarity = 0.306.

are displaced due to their high iron concentrations (Table 5). Sample PG 8 (unpulped ripe cherries) was displaced towards Fe, Na, Ca, and K, confirming the nutritional value of the coffee rinds in manuring applications and production of animal foodstuffs. The HCA (Figure 2) confirmed similarities existing among elemental levels and PCA results.

## Conclusions

The *t*-test showed that results obtained by digester heating block procedure were in agreement at 95% confidence level with those obtained by the microwave oven procedure for Cu, Mn, K, and Mg. Digester heating block system is susceptible to Na and Zn contamination. On the other hand, this system led to lower Ca concentration for most samples. Both procedures can be used for determination of Fe in green coffee samples. For roasted coffee, only microwave oven was effective. The Willye-type mill contaminated the coffee samples with Fe. High quantities of Na, Ca, Fe, and K were found in coffee rinds. These can be used as a good source of inorganic nutrients (Ca, Fe, and K) in fertilizers and animal feeding.

The use of Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) applied to the inorganic composition of coffee allowed to observe similarities and discrepancies between green and roasted coffees. In general, roasted coffees present higher levels of Na, K, Ca, Mg, and Fe. The main elements responsible for the discrimination between green and roasted coffee samples were Na, K, Ca, Mg, Fe, and Cu.

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## Supplementary Information

The results obtained for the 35 analyzed samples are available free of charge at <http://jbcs.s bq.org.br>, as PDF file.

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