

## Multivariate Analysis: Use of Doehlert Design (DD) for Optimization of a Simple Sample Preparation Procedure for the Determination of Inorganic Constituents in Cocoa Beans Employing Microwave Acid Digestion and ICP OES

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This study presents a microwave-assisted procedure for sample preparation of cocoa beans using diluted HNO<sub>3</sub> with H<sub>2</sub>O<sub>2</sub> for the determination of Ba, Ca, Cu, K, Mg, Mn, P, S, Sr and Zn by inductively coupled plasma optical emission spectrometry (ICP OES). Doehlert modified design was used for optimization of experimental condition, and the volume of H<sub>2</sub>O<sub>2</sub> (0, 0.5, 1.75 and 3 mL) and concentration of HNO<sub>3</sub> (0.5, 3.75 and 7 mol L<sup>-1</sup>) were evaluated. The best experimental conditions were chosen considering lower residual acidity (RA), lower dissolved organic carbon (DOC) and the acceptable trueness range (80-120%), were 5 mL of 3.75 mol L<sup>-1</sup> HNO<sub>3</sub> and 1.75 mL of 30% v v<sup>-1</sup> H<sub>2</sub>O<sub>2</sub>. The procedure was validate using certified reference materials (CRMs), digested using the optimized conditions. The proposed procedure was applied in twelve samples of cocoa beans, and the concentrations (mg kg<sup>-1</sup>) ranged from: 7.6-27 (Ba), 520-1955 (Ca), 11-26 (Cu), 6212-10855 (K), 1677-2516 (Mg), 13-29 (Mn), 4679-6104 (P), 1499-1898 (S), 7-32 (Sr) and 34-46 (Zn). A Pearson correlation plot was proposed to evaluate the correlation between elements, revealing high correlations between Mg and K; K and P; Cu and Mn; Cu and Zn; Cu and S.

**Keywords:** Doehlert design, microwave-assisted digestion, diluted acid, cocoa bean from southern of Bahia

### Introduction

According to the International Cocoa Organization (ICO), the southern America region is the second major producer worldwide of cocoa bean (*Theobroma cacao* L.), with approximately 18% of the total.<sup>1,2</sup> Currently, Brazil is the seventh largest producer of cocoa beans with approximately 200 thousand tons *per year*.<sup>1,2</sup> In this scenario, the southern region of Bahia State is responsible for the majority of the production of cocoa bean in Brazil.<sup>1,2</sup> However, despite the significant contribution of this region in the production of cocoa bean, the data about the chemical

composition of these samples are still insufficient or even absent in the literature.

It is reported in the scientific literature that the cocoa bean and its derivates have high levels of bioactive natural constituents (methylxanthines and phenolic compounds) and antioxidants that are beneficial compounds for human health.<sup>3-6</sup> These compounds minimize the harmful effects caused by free radicals in the human body. In addition, it contributes to the reduction of cardiovascular disease and cancer risk.<sup>7,8</sup> Cocoa bean and its derivatives, are also an important source of nutrients, such as Ca, Cu, K, Mg, Mn and P.<sup>9,10</sup> However, some studies<sup>11-14</sup> also reported the presence of potentially toxic elements, such as As, Ba, Cd, Cr, Ni, Pb and Sb. Therefore, the elemental composition

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of cocoa beans should be monitored constantly in order to determine its chemical elements constituents.

Commonly, the determination of nutrients and potentially toxic elements in cocoa beans and its derivatives are performed by different analytical techniques, such as graphite furnace atomic absorption spectrometry (GF AAS), inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma mass spectrometry (ICP-MS).<sup>9-14</sup> Nonetheless, these techniques are susceptible to severe matrix effects, which may influence the precision and accuracy of the analysis. These effects may be more severe in cocoa beans and its derivatives, due to peculiar characteristics of the matrix, as high fat content.<sup>6</sup>

One possible strategy to overcome matrix effects problems is the development of an efficient sample preparation method.<sup>15</sup> In this sense, microwave-assisted digestion with closed vessels using dilute nitric acid (HNO<sub>3</sub>) has been employed as an effective strategy for decomposition of complex samples, i.e., that contain high quantities of fats and oils, with similar results to those obtained after decomposition with concentrated acids.<sup>16-19</sup> In addition, the use of dilute HNO<sub>3</sub> presents some advantages, such as reduced analytical blank signal, which is correlated to lower limits of detection. Moreover, generates less aggressive waste, following green chemistry principles.<sup>20</sup>

In recent years, multivariate tools have been widely applied in analytical chemistry for the optimization of sample preparation methods of food samples.<sup>10,21-24</sup> Doehlert design (DD) is a chemometric tool mostly used in analytical chemistry, and present advantages over others for response surface methodology (RSM), allowing: (i) estimate the parameters of the quadratic model, (ii) building of sequential designs, (iii) detection of lack of fit of the model, and (iv) use of blocks.<sup>25,26</sup>

In this study, a microwave-assisted digestion procedure using dilute HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> were used for multi-elemental determination of Ba, Ca, Cu, K, Mg, Mn, P, S, Sr and Zn in cocoa beans samples produced in Bahia. Additionally, the variables evaluated in the microwave-assisted digestion procedure were optimized using DD. Therefore, this study is devoted to development of a simple, fast and green sample preparation procedure for evaluation of the elemental composition of cocoa beans.

## Experimental

### Instrumentation

The determination of Ba, Ca, Cu, K, Mg, Mn, P, S, Sr

and Zn was carried out using an inductively coupled plasma optical emission spectrometer (ICP OES) iCAP 7000 (Thermo Fisher, Madison, WI, USA). The measurements were performed using argon gas 99.999% (White Martins-Praxair, Sertãozinho, SP, Brazil), utilizing both axial and radial viewing modes. The instrumental conditions and the emission lines used are shown in Table 1.

The sample decomposition was performed using a single reaction chamber oven (UltraWave™, Milstone, Sorisole, Italy). Before digestion process, a volume of 150 mL of water and 5.0 mL of concentrated HNO<sub>3</sub> were added into the single reaction chamber, which was pressurized with nitrogen gas (99.9%, White Martins-Praxair, Sertãozinho, Brazil) until 40 bar.

**Table 1.** Instrumental parameters for ICP OES determinations

Parameter	ICP OES
Radio frequency applied power / kW	1.2
Plasma gas flow rate / (L min <sup>-1</sup> )	12.0
Auxiliary gas flow rate / (L min <sup>-1</sup> )	0.5
Nebulizer gas flow rate / (L min <sup>-1</sup> )	0.50
Analyte	Emission line, λ / nm
Ba	455.403 (II)
Ca	393.366 (II)
Cu	324.754 (I)
K	766.490 (I)
Mg	280.270 (II)
Mn	259.373 (II)
P	177.495 (I)
S	182.034 (I)
Sr	421.552 (II)
Zn	213.856 (I)

I: atomic emission line, II: ionic emission line. ICP OES: inductively coupled plasma optical emission spectrometry.

### Standards, reagents and samples

Analytical-grade reagents and deionized water (Milli-Q system, 18.2 MΩ cm, Millipore, Bedford, MA, USA) were used to prepare all solutions and standards. All glassware and vessels were soaked in a 10% v v<sup>-1</sup> HNO<sub>3</sub> (Synth, Diadema, SP, Brazil) solution during 24 h. After that, a rinsing step with deionized water was performed, and the materials were left to dry in a clean hood before use. The multielementar solutions used for external calibration were prepared from dilution of 1000 mg L<sup>-1</sup> of Ba, Ca, Cu, K, Mg, Mn, P, S, Sr and Zn standard solutions (Qhemis, São Paulo, SP, Brazil) using dilute HNO<sub>3</sub>, i.e., 1% v v<sup>-1</sup> (Synth, Diadema, SP, Brazil), previously purified using a

sub-boiling distillation system Distillacid™ BSB-939-IR (Berghof, Eningen, Germany).

A total of twelve cocoa bean samples coded as S1 to S12 were obtained in an area of the Cocoa Research Center (CEPEC), an entity linked to the Executive Committee of the Cacao Plan (CEPLAC) of the Brazilian Ministry of Agriculture, Livestock and Supply (MAPA) localized in Bahia State. The samples were dried and grinded using a mortar and pestle to reduce the particle size and was not necessary to mash before digestion. In the case of fat content, the acquired samples did not present an enough quantity that could affect the process of sample preparation and further determination of inorganic constituents in cocoa beans.

In addition, four certified reference materials (CRMs) of baking chocolate (NIST 2384), apple leaves (NIST 1515), spinach leaves (NIST 1570a), and whole milk powder (NIST 8435), all from National Institute of Standard and Technology (Gaithersburg, MD, USA), were used to evaluate the efficiency of sample preparation method with diluted HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>.

#### Multivariate optimization of the digestion procedure

The optimization of the digestion process for the sample preparation was performed using a Doehlert modified design. To carry out the optimization step, one sample of cocoa bean was chosen; moreover, some variables were evaluated, such as HNO<sub>3</sub> concentration in three levels (0.5, 3.75 and 7 mol L<sup>-1</sup>), and the volume of H<sub>2</sub>O<sub>2</sub> in four

levels (0, 0.5, 1.75 and 3 mL), as depicted in Table 2. For this, a total of 14 experiments were performed including 3 replicates at the central point in order to evaluate the experimental error. All data organization and calculations were performed using MATLAB 2018a.<sup>27</sup>

The optimal conditions for all elements were chosen considering the acceptable trueness (in percentage) calculated from the proposed method and the reference method using concentrated HNO<sub>3</sub>. Moreover, lower residual acidity and dissolved organic carbon were evaluated. In this sense, the multi-response optimization proposed by Candioti *et al.*<sup>28</sup> was taken in account, i.e., the criteria was to obtain trueness (in percentage) between 80 and 120% of each analyte and minimize both the residual acidity (RA, in % v v<sup>-1</sup>) and dissolved organic carbon (DOC, in mg L<sup>-1</sup>). RA was calculated after titration of digested solutions with standardized NaOH solution. DOC was evaluated after the determination of C in the digested samples employing ICP OES. Oxalic acid was utilized to build the calibration curve for C determinations.

#### Microwave-assisted sample preparation

A mass of 200 mg of cocoa beans was directly weighed in perfluoroalkoxy alkanes (PFA) vessels and, 5 mL of 3.75 mol L<sup>-1</sup> HNO<sub>3</sub> and 1.75 mL of 30% v v<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> were added. The samples were submitted to the following heating program (temperature in °C/ramp in min/hold in min): (i) 180/5/5; (ii) 210/5/10; (iii) 230/5/10, and the limit

**Table 2.** Matrix of DD and results (trueness, RA, dissolved organic carbon and overall desirability, OD) for the optimization of sample preparation

Exp	Variable		Trueness / %										RA / (% v v <sup>-1</sup> )	DOC / (mg L <sup>-1</sup> )	OD
	H <sub>2</sub> O <sub>2</sub> / mL	HNO <sub>3</sub> / (mol L <sup>-1</sup> )	Ca	K	Mg	P	S	Mn	Zn	Sr	Cu	Ba			
1	1.75	3.75	129	135	116	91	111	98	100	24	69	36	3.45	384	0.6
2	1.75	3.75	119	118	108	100	111	111	104	94	101	81	3.45	247	1.0
3	1.75	3.75	113	120	107	86	107	121	99	94	93	81	3.45	459	0.9
4	0.5	3.75	113	119	106	89	106	124	97	94	41	80	2.51	461	0.9
5	3	3.75	128	129	113	91	110	123	102	102	96	88	4.39	683	0.9
6	0.5	7	108	110	103	86	103	164	97	91	84	80	5.65	266	0.8
7	1.75	7	118	118	107	89	105	92	99	97	89	84	7.06	624	0.9
8	3	7	110	113	102	86	101	89	94	93	84	81	7.53	597	0.9
9	0.5	0.5	114	127	111	96	104	126	84	101	81	91	0.20	655	0.9
10	1.75	0.5	124	137	117	116	124	130	110	117	95	103	0.28	1246	0.8
11	3	0.5	99	111	99	97	102	114	95	96	80	88	0.28	1660	0.9
12	0	0.5	111	119	99	93	104	113	90	88	48	66	0.09	723	0.8
13	0	3.75	105	108	100	89	104	117	99	88	87	76	2.20	490	1.0
14	0	7	113	120	105	93	106	111	98	95	89	82	5.14	100	0.9

Exp: experiment; RA: residual acidity; DOC: dissolved organic carbon.

pressure of the digestion method was established as 110 bar. After that, the vessel content was transferred to volumetric flasks and the solution was diluted to 25 mL with ultrapure water before analysis.

## Results and Discussion

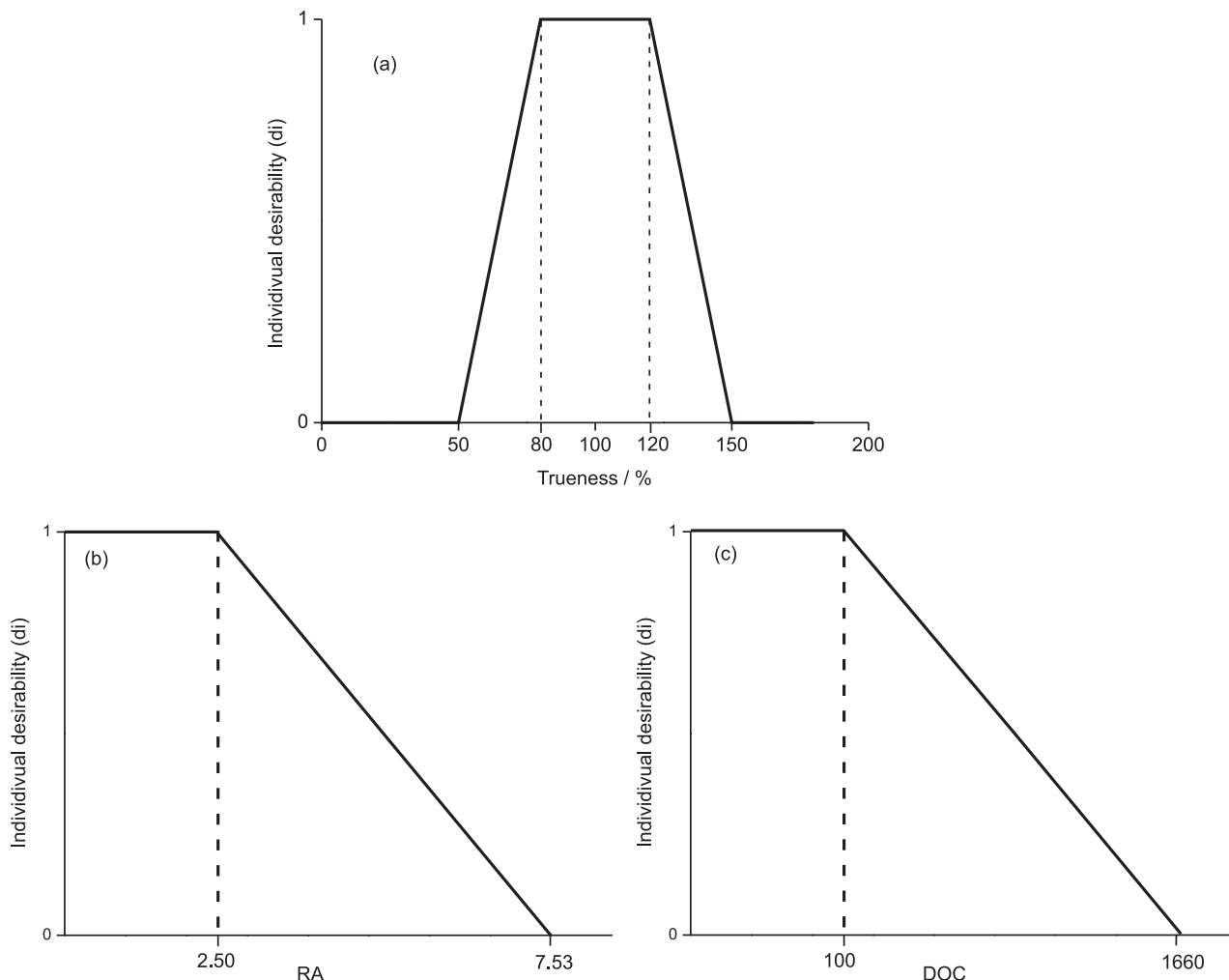
### Optimization of microwave-assisted digestion

A modified DD was used to adapt the coded and experimental conditions for practicality purpose, in order to optimize the best conditions of microwave-assisted digestion using diluted HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>. Table 2 shows the performed experiments, as well as the trueness values obtained for Ba, Ca, Cu, K, Mg, Mn, P, S, Sr and Zn. In addition, Table 2 displays the values of RA and DOC obtained for each experimental condition. The procedure was optimized using one sample of cocoa bean, and 14 experiments including three replicates in the central point (experiments 1, 2 and 3) were performed randomly.

To obtain a single experimental condition, overall desirability (OD, see Table 2) was calculated to ensure the compliance criteria using all responses simultaneously. The first step was to calculate the individual desirability (*d<sub>i</sub>*) for each response, which implies that each response is converted to a value coded from 0 (undesired response) to 1 (desired response). Figure 1 shows a schematic diagram of individual desirability according to different criteria: trueness values (Figure 1a), RA (v v<sup>-1</sup>) (Figure 1b), and DOC (mg L<sup>-1</sup>) (Figure 1c). In this sense, the *d<sub>i</sub>* = 1 was attributed for the elements that presented the trueness from 80 to 120%, RA below 2.5 v v<sup>-1</sup> and DOC below 100 mg L<sup>-1</sup>. However, for the responses that present trueness values between 50 and 80, the *d<sub>i</sub>* was calculated using equation 1.

$$d_i = \left( \frac{y - L}{T - L} \right)^s \quad (1)$$

The *y* corresponds to the response of the experiment, *L* (lower) and *T* (target) corresponds to 50 and 80%,



**Figure 1.** Schematic diagram of individual desirability calculation according to the following criteria: (a) trueness values, (b) RA, and (c) DOC.

respectively,  $s$  is the weight used for calculation and in the specific case of this study was 1.

On the other hand, the  $d_i$  for the responses that presents trueness values between 120 and 150%, RA between 2.50 and 7.53  $v v^{-1}$  and DOC between 100 and 1660  $mg L^{-1}$  was calculated using equation 2.

$$d_i = \left( \frac{U - y}{U - T} \right)^t \quad (2)$$

where the  $y$  is the response of the experiment,  $U$  (high value) equals to trueness of 150%, RA 7.53  $v v^{-1}$  and DOC 1660  $mg L^{-1}$ ;  $T$  (target) corresponds to trueness of 120%, RA 2.50  $v v^{-1}$ , DOC 100  $mg L^{-1}$ , and  $t$  is the weight used for calculation and in the specific case of this study was 1. While  $d_i = 0$  for responses that are outside of the acceptable trueness values, i.e., below of 50% and higher than 150%, RA higher than 7.53  $v v^{-1}$ , and DOC higher than 1660  $mg L^{-1}$ .

After the  $d_i$  calculations for each response, the OD was calculated using the arithmetic mean of  $d_i$  of each experiment, as can be shown in Table 2. Arithmetic mean was used in this case in order to avoid 0 values for OD. After that, a mathematical model was generated and evaluated by analysis of variance (ANOVA) at 95% of confidence level. Thus, the model obtained does not presented lack of fit because the ratio between the mean square of lack of fit (MSlof) and mean square of pure error (MSpe), the  $F_{\text{calculated}}$  (0.194) was lower than  $F_{\text{tabulated}}$  (19.3) at 95% confidence level. However, comparing the mean of square of regression (MSR) and mean of square of residue (MSres), the  $F_{\text{calculated}}$  (0.118) should be higher than  $F_{\text{tabulated}}$  (3.69). In this sense, it showed that the regression was not good enough, however, it does not affect the capability of the model in order to present a conclusion about the most convenient experimental condition. This design generated the OD calculated from equation 3, and only the constant (b0) in bold was significant.

$$OD = \mathbf{0.854} \pm \mathbf{0.137} - 0.002 \pm 0.104v_1 + 0.014 \pm 0.117v_2 + 0.070 \pm 0.179v_{11} - 0.047 \pm 0.211v_{22} - 0.001 \pm 0.150v_1v_2 \quad (3)$$

$v_1$  and  $v_2$  are linear coefficients for variables 1 ( $H_2O_2$ ) and 2 ( $HNO_3$ ), respectively,  $v_{11}$  and  $v_{22}$  are quadratic coefficients for variables 1 and 2, respectively and  $v_1v_2$  is the interaction coefficient.

As can be noted from equation 3, the  $HNO_3$  concentration and volume of  $H_2O_2$  were not significant. In this sense, any experimental domain can be used for the sample preparation procedure. In our specific case, the optimized values

considered ideal and used for further sample preparation were 3.75  $mol L^{-1}$  of  $HNO_3$  and 1.75 mL of  $H_2O_2$  (central point), in addition showed satisfactory values of RA, 3.45%  $v v^{-1}$  and DOC 247  $mg L^{-1}$ .

It is important to emphasize that in some cases milder experimental conditions could be used; however, the decompositions were not efficient since the RA and/or DOC were high according to the results shown in Table 2 (experiments 4, 6, 9, and 10-13).

Analytical characteristics of the developed method and application

For the determination of Ba, Ca, Cu, K, Mg, Mn, P, S, Sr and Zn in cocoa beans, several analytical characteristics were evaluated. In this sense, the limit of detection (LOD) and quantification (LOQ) of the developed procedure were calculated considering three-fold and ten-fold standard deviation (SD) from ten consecutive measurements of the analytical blank. Table 3 shows the LOD and LOQ values for the developed procedure. It is possible to observe that these values are sufficiently lower, reflecting in good sensitivity of the method. Moreover, the precision was also evaluated considering the relative standard deviation (RSD, in percentage) for 5 authentic replicates digestions of a single sample using diluted  $HNO_3$  (3.75  $mol L^{-1}$ ) and 1.75 mL of  $H_2O_2$ , and the results are depicted in Table 3.

**Table 3.** Limits of detection (LOD) and quantification (LOQ), and relative standard deviation (RSD) obtained for proposed procedure

Element	Proposed procedure		
	LOD / ( $mg kg^{-1}$ )	LOQ / ( $mg kg^{-1}$ )	RSD / %
Ba	0.1	0.4	6
Ca	5	17	8
Cu	0.5	2	4
K	2	5	1
Mg	0.5	2	5
Mn	0.2	0.5	4
P	196	653	2
S	13	43	1
Sr	0.05	0.2	7
Zn	1	4	7

For the evaluation of the proposed procedure, four different CRMs, such as apple and spinach leaves, baking chocolate and whole milk powder were submitted to the optimized conditions described in previous sub-section "Optimization of microwave-assisted digestion", and the results are shown in Table 4. The results obtained using



this procedure demonstrated that the concentration of the analytes in the majority of CRM are in agreement with certified values according to Student's *t*-test at 95% of confidence level. Some exceptions were observed and are marked as superscript "b". Nevertheless, in all cases presented acceptable trueness values (80-120%), except for S in apple leaves. Moreover, the results obtained employing the total digestion of the samples with concentrated HNO<sub>3</sub> and with proposed procedure were also compared using paired *t*-test at 95% of confidence level. The results showed that there is no significant difference between the reference method using concentrated HNO<sub>3</sub> and the proposed procedure using diluted HNO<sub>3</sub>.

Therefore, the optimized conditions were used to determine the concentration levels of Ba, Ca, Cu, K, Mg, Mn, P, S, Sr and Zn in twelve different cocoa bean samples from Bahia, Brazil, and the results are shown in Table 5. The concentration values using the proposed method ranged from 7.6 to 27 mg kg<sup>-1</sup> for Ba, 520 to 1955 mg kg<sup>-1</sup> for Ca, 11 to 26 mg kg<sup>-1</sup> for Cu, 6212 to 10855 mg kg<sup>-1</sup> for K, 1677 to 2516 mg kg<sup>-1</sup> for Mg, 13 to 29 mg kg<sup>-1</sup> for Mn, 4679 to 6104 mg kg<sup>-1</sup> for P, 1499 to 1898 mg kg<sup>-1</sup> for S, 7 to 32 mg kg<sup>-1</sup> for Sr and 34 to 46 mg kg<sup>-1</sup> for Zn.

From the results, is possible to note that Ca, K, Mg, P and S are major constituents in all samples, while Ba, Cu, Mn, Ni, Sr and Zn are found at lower levels. Due to nutritional reasons, the determination of chemical constituents in this type of sample is important, once cocoa bean samples are used as raw material for production of cocoa powder, chocolates and other products used in the human diet.

How the chemical elements determined by ICP OES are correlated in the samples?

In this section, we intend to evaluate the correlation among the elements and, at the same time explain why it occurs. For this purpose, the concentration values of the determined elements were organized in a matrix **X** (10 × 12), where the columns and rows correspond to the elements (10 analytes) and their concentrations at the samples (12), respectively. After that, the data were subjected to an exploratory analysis employing the Pearson correlation coefficient (R). This procedure measures the linear correlation degree between the elements determined by ICP OES technique.

To explain the possible correlation in the samples, a Pearson correlation plot was generated. In addition, each value obtained from the correlation coefficients is related to specific color, i.e., the highest absolute value near to 1 indicates or reflects a higher intensity of linear relationship among elements.

Figure 2 shows the Pearson correlation plot used to correlate the elements in the sample. Blue and red color represents the low and high correlation between elements, respectively. In addition, the correlation of a determined element with itself is always equal to 1.

As an example of this correlation, according to Figure 2, Mg and K are highly correlated in the samples, which means that, when the concentration of Mg increases, the concentration values of K also increases simultaneously. The same behavior can be observed for other chemical elements such as K and P, Cu and Mn, Cu and Zn, and Cu

**Table 4.** Concentration values of Ca, Cu, Ba, K, Mg, Mn, P, S, Sr and Zn obtained in certified reference materials using proposed procedure (mean ± standard deviation, n = 3 and trueness)

Element	Concentration / (mg kg <sup>-1</sup> )							
	Baking chocolate (NIST 2384)		Apple leaves (NIST 1515)		Spinach leaves (NIST 1570a)		Whole milk powder (NIST 8435)	
	Certified value	Proposed procedure (trueness)	Certified value	Proposed procedure (trueness)	Certified value	Proposed procedure (trueness)	Certified value	Proposed procedure (trueness)
Ba	<sup>a</sup>	–	49 ± 2	48.8 ± 0.2 (100)	<sup>a</sup>	–	<sup>a</sup>	–
Ca	840 ± 74	865.9 ± 0.2 (103) <sup>b</sup>	15250 ± 100	14147 ± 126 (93) <sup>b</sup>	15270 ± 410 <sup>b</sup>	14275 ± 52 (94) <sup>b</sup>	9220 ± 490	9966 ± 445 (108) <sup>b</sup>
Cu	24 ± 1	21.9 ± 0.5 (91) <sup>b</sup>	5.7 ± 0.1	5.1 ± 0.5 (90)	12.2 ± 0.6	14 ± 1 (115)	0.46 ± 0.08	0.42 ± 0.02 (91)
K	8650 ± 400	7120 ± 17 (82) <sup>b</sup>	16080 ± 210	12821 ± 198 (80) <sup>b</sup>	29030 ± 520	24053 ± 760 (82) <sup>b</sup>	13630 ± 470	10746 ± 139 (79) <sup>b</sup>
Mg	2610 ± 120	2624 ± 101 (101)	2710 ± 120	2649 ± 33 (98)	<sup>a</sup>	–	814 ± 76	835 ± 3 (103) <sup>b</sup>
Mn	21 ± 1	21.7 ± 0.4 (103)	54 ± 1	46 ± 3 (85) <sup>b</sup>	76 ± 2	75 ± 5 (99)	0.17 ± 0.05	0.18 ± 0.01 (106)
P	3330 ± 210	3333 ± 32 (100)	1593 ± 68	1781 ± 21 (112) <sup>b</sup>	5180 ± 110	5059 ± 153 (98)	7800 ± 490	7411 ± 138 (95) <sup>b</sup>
S	<sup>a</sup>	–	1800 <sup>c</sup>	1279 ± 72 (71)	4600 <sup>c</sup>	4818 ± 142 (105)	2650 ± 350	2346 ± 56 (86) <sup>b</sup>
Sr	<sup>a</sup>	–	25 ± 1	24.9 ± 0.3 (100)	55.6 ± 0.8	54.6 ± 0.5 (98)	4.3 ± 0.5	4.3 ± 0.1 (100)
Zn	38 ± 2	37 ± 1 (97)	12.4 ± 0.5	13.2 ± 0.1 (107) <sup>b</sup>	82 ± 3	76 ± 1 (93) <sup>b</sup>	28 ± 3	27 ± 0.5 (97)

<sup>a</sup>Value not informed; <sup>b</sup>the obtained value was not statistically concordant with certified value using Student's *t*-test at 95% of confidence level; <sup>c</sup>informative value.

**Table 5.** Comparison among the concentration values obtained using microwave digestion with proposed procedure and concentrated acid in the sample preparation for the determination of Ca, Cu, Ba, K, Mg, Mn, P, S, Sr and Zn in cocoa beans (mean  $\pm$  standard deviation, n = 3 and trueness)

Sample	Sample preparation	Concentration / (mg kg <sup>-1</sup> )									
		Ba	Ca	Cu	K	Mg	Mn	P	S	Sr	Zn
S1	proposed procedure	25 $\pm$ 1	1125 $\pm$ 22	11.9 $\pm$ 0.3	9727 $\pm$ 503	2516 $\pm$ 27	21.5 $\pm$ 0.6	6100 $\pm$ 284	1546 $\pm$ 22	31.6 $\pm$ 0.9	41 $\pm$ 1
	concentrated acid	23.5 $\pm$ 0.3	1070 $\pm$ 15	11.60 $\pm$ 0.06	8030 $\pm$ 68	2589 $\pm$ 7	20.25 $\pm$ 0.03	8628 $\pm$ 55	1465 $\pm$ 3	29.27 $\pm$ 0.05	40 $\pm$ 1
S2	proposed procedure	13.0 $\pm$ 0.3	520 $\pm$ 13	21.0 $\pm$ 0.5	7340 $\pm$ 290	2299 $\pm$ 8	22.6 $\pm$ 0.3	5029 $\pm$ 122	1712 $\pm$ 13	13.2 $\pm$ 0.1	43 $\pm$ 10
	concentrated acid	12.0 $\pm$ 0.3	474 $\pm$ 7	18.1 $\pm$ 0.8	5663 $\pm$ 95	2219 $\pm$ 77	20.5 $\pm$ 0.9	5106 $\pm$ 158	1457 $\pm$ 54	12.0 $\pm$ 0.2	40.8 $\pm$ 0.2
S3	proposed procedure	16.8 $\pm$ 0.2	690 $\pm$ 30	26 $\pm$ 1	9730 $\pm$ 569	2452 $\pm$ 102	29 $\pm$ 1	6104 $\pm$ 210	1681 $\pm$ 33	12.1 $\pm$ 0.3	43 $\pm$ 1
	concentrated acid	13.6 $\pm$ 0.4	560 $\pm$ 28	20 $\pm$ 1	6929 $\pm$ 25	2133 $\pm$ 104	24 $\pm$ 1	6572 $\pm$ 237	1426 $\pm$ 16	9.5 $\pm$ 0.3	36.7 $\pm$ 0.9
S4	proposed procedure	9.0 $\pm$ 0.6	694 $\pm$ 52	16.9 $\pm$ 0.7	8830 $\pm$ 111	2240 $\pm$ 100	15 $\pm$ 1	5219 $\pm$ 125	1637 $\pm$ 13	9 $\pm$ 1	45 $\pm$ 1
	concentrated acid	8.4 $\pm$ 0.8	618 $\pm$ 8	15.7 $\pm$ 0.4	6601 $\pm$ 55	2078 $\pm$ 51	12.6 $\pm$ 0.3	6806 $\pm$ 33	1513 $\pm$ 11	8.1 $\pm$ 0.2	42 $\pm$ 1
S5	proposed procedure	10.6 $\pm$ 0.3	886 $\pm$ 34	15.5 $\pm$ 0.4	10855 $\pm$ 284	2406 $\pm$ 16	14.4 $\pm$ 0.2	5689 $\pm$ 101	1635 $\pm$ 6	12.0 $\pm$ 0.3	40.0 $\pm$ 0.2
	concentrated acid	10 $\pm$ 1	803 $\pm$ 76	15 $\pm$ 2	8815 $\pm$ 600	2394 $\pm$ 215	13.0 $\pm$ 0.9	5993 $\pm$ 161	1472 $\pm$ 38	11 $\pm$ 1	36 $\pm$ 2
S6	proposed procedure	21.7 $\pm$ 0.4	921 $\pm$ 42	25 $\pm$ 2	10292 $\pm$ 751	2491 $\pm$ 101	24 $\pm$ 2	5892 $\pm$ 449	1898 $\pm$ 66	13.9 $\pm$ 0.3	45 $\pm$ 3
	concentrated acid	20 $\pm$ 2	897 $\pm$ 85	23 $\pm$ 2	9228 $\pm$ 168	2557 $\pm$ 239	22 $\pm$ 1	8611 $\pm$ 615	1853 $\pm$ 63	13 $\pm$ 1	44 $\pm$ 4
S7	proposed procedure	17.7 $\pm$ 0.4	840 $\pm$ 17	20.78 $\pm$ 0.04	9707 $\pm$ 469	2397 $\pm$ 16	26.5 $\pm$ 0.4	6101 $\pm$ 311	1711 $\pm$ 51	12.1 $\pm$ 0.1	44 $\pm$ 0.6
	concentrated acid	15.9 $\pm$ 0.3	791 $\pm$ 50	18.8 $\pm$ 0.2	8010 $\pm$ 231	2424 $\pm$ 115	25 $\pm$ 1	6404 $\pm$ 387	1482 $\pm$ 15	11.2 $\pm$ 0.5	40 $\pm$ 2
S8	proposed procedure	12.2 $\pm$ 0.5	555 $\pm$ 25	20 $\pm$ 1	7909 $\pm$ 687	2010 $\pm$ 103	15.4 $\pm$ 0.7	4720 $\pm$ 150	1688 $\pm$ 42	7.4 $\pm$ 0.5	39 $\pm$ 1
	concentrated acid	11.2 $\pm$ 0.4	517 $\pm$ 10	19.2 $\pm$ 0.5	7018 $\pm$ 97	1996 $\pm$ 26	14.59 $\pm$ 0.07	6183 $\pm$ 10	1614 $\pm$ 11	6.9 $\pm$ 0.1	36.3 $\pm$ 0.7
S9	proposed procedure	25.2 $\pm$ 0.4	622 $\pm$ 7	15.3 $\pm$ 0.3	6212 $\pm$ 245	1677 $\pm$ 27	14.7 $\pm$ 0.4	4679 $\pm$ 10	1521 $\pm$ 6	15.6 $\pm$ 0.2	42.1 $\pm$ 0.4
	concentrated acid	22 $\pm$ 2	540 $\pm$ 47	13 $\pm$ 2	4811 $\pm$ 367	1572 $\pm$ 169	13 $\pm$ 2	4800 $\pm$ 404	1293 $\pm$ 85	13 $\pm$ 1	38 $\pm$ 3
S10	proposed procedure	27.0 $\pm$ 0.4	703 $\pm$ 6	18 $\pm$ 1	7403 $\pm$ 123	1967 $\pm$ 17	25 $\pm$ 1	5058 $\pm$ 136	1589 $\pm$ 40	13.1 $\pm$ 0.2	46 $\pm$ 1
	concentrated acid	26 $\pm$ 1	678 $\pm$ 46	17 $\pm$ 1	6719 $\pm$ 240	2097 $\pm$ 14	25 $\pm$ 1	6366 $\pm$ 111	1428 $\pm$ 70	12.7 $\pm$ 0.3	44 $\pm$ 3
S11	proposed procedure	7.6 $\pm$ 0.2	1955 $\pm$ 284	12.6 $\pm$ 0.1	9367 $\pm$ 803	2177 $\pm$ 31	13.1 $\pm$ 0.2	5755 $\pm$ 240	1545 $\pm$ 24	13 $\pm$ 1	34 $\pm$ 2
	concentrated acid	6.95 $\pm$ 0.03	1880 $\pm$ 9	11.60 $\pm$ 0.01	7576 $\pm$ 65	2206 $\pm$ 4	12.5 $\pm$ 0.3	6308 $\pm$ 3	1376 $\pm$ 16	9.4 $\pm$ 0.1	32.2 $\pm$ 0.7
S12	proposed procedure	23 $\pm$ 1	584 $\pm$ 11	11.3 $\pm$ 0.2	7274 $\pm$ 453	1871 $\pm$ 32	15.1 $\pm$ 0.2	5261 $\pm$ 276	1499 $\pm$ 4	23 $\pm$ 1	33.6 $\pm$ 0.2
	concentrated acid	20 $\pm$ 3	524 $\pm$ 58	10 $\pm$ 2	5798 $\pm$ 380	1932 $\pm$ 225	13 $\pm$ 2	5892 $\pm$ 519	1344 $\pm$ 56	20 $\pm$ 2	29 $\pm$ 4

and S which presents high correlation and converge in or near of red color.

There was noticed an inversely correlation with elements Ba, Ca, K, S and Sr, i.e., when the concentration values of these elements increase, the concentration values

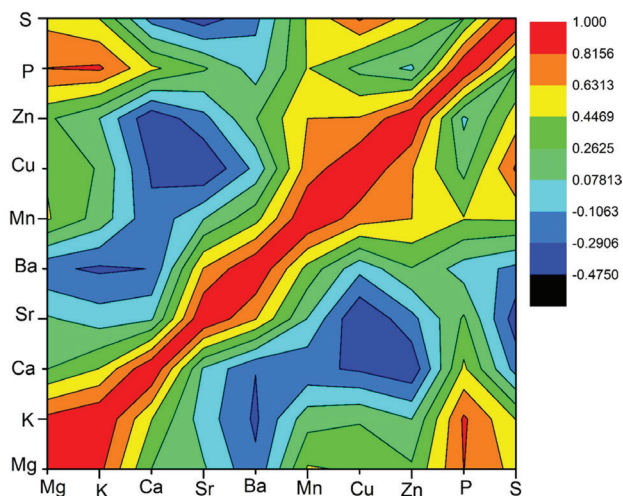
of other elements decrease. However, the lower or higher correlation between elements in the samples can be related to the type of fertilizer used in the soil fortification.

A study performed by Jiao *et al.*<sup>29</sup> related to the growth and nutrient response of flax and durum wheat to P and Zn

fertilizers or mixture of them, shows how application of P and Zn fertilizers on the nutrient composition can affect the translocation of nutrients to the seed or grain.

The authors explain that, for example, P fertilizers enhanced Fe but reduced Ca and S, although increased K translocation to the seed and grain. While Zn fertilizers had no effect on K, enhanced Ca, but decrease S and Fe in grain. Therefore, the results obtained in present study shows a similar tendency if we assumed that the production of these seeds were based on P and Zn fertilizers.

Therefore, it is well known that, in the cocoa beans production, P and Zn fertilizers have been used to fill the deficiencies of these elements in order to improve the soil fertility. As shown in Figure 2, for example, high content of P can enhance the Mg and K concentration, but also reduce Ca and S content; however, the high content of Zn fertilizers can prevent the absorption of Ca, K and P content. Thus, the results showed in the Pearson correlation plot can be related to the type of fertilizers used and soil, moreover, also depends on the type of culture.



**Figure 2.** Pearson plot used to evaluate the correlation (R) of the elements in the samples.

## Conclusions

The use of DD for optimization of microwave-assisted digestion showed to be effective for sample preparation of cocoa beans for the determination of micro and macro nutrients using  $3.75 \text{ mol L}^{-1}$  of  $\text{HNO}_3$  (diluted) and  $1.75 \text{ mL}$  of  $\text{H}_2\text{O}_2$ . The proposed procedure showed the best results, where the trueness values are in the acceptable range. Moreover, combining multivariate optimization and other chemometric tools, was possible to evaluate the higher and lower correlation of the elements in the samples using Pearson correlation plot.

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## Author Contributions

Raimundo R. Gamela was responsible for formal analysis, conceptualization; visualization; roles/writing-original draft; writing-review and editing; Vinicius C. Costa for formal analysis; visualization, roles/writing-original draft, writing-review and editing; Matheus L. de Mello for formal analysis, conceptualization, visualization, roles/writing-original draft; Julia C. Romero for formal analysis; Fábio A. C. Amorim for formal analysis; Edenir R. P. Filho for formal analysis, funding acquisition, project administration, resources, supervision, visualization, writing-review and editing.

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