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Multivariate optimization of an analytical method for the analysis of dog and cat foods by ICP OES

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ABSTRACT

Experimental design methodology was used to optimize an analytical method for determination of the mineral element composition (Al, Ca, Cd, Cr, Cu, Ba, Fe, K, Mg, Mn, P, S, Sr and Zn) of dog and cat foods. Two-level full factorial design was applied to define the optimal proportions of the reagents used for microwave-assisted sample digestion (2.0 mol L^{-1} HNO_3 and 6% m/v H_2O_2). A three-level factorial design for two variables was used to optimize the operational conditions of the inductively coupled plasma optical emission spectrometer, employed for analysis of the extracts. A radiofrequency power of 1.2 kW and a nebulizer argon flow of 1.0 L min^{-1} were selected. The limits of quantification (LOQ) were between 0.03 μg g^{-1} (Cr, 267.716 nm) and 87 μg g^{-1} (Ca, 373.690 nm). The trueness of the optimized method was evaluated by analysis of five certified reference materials (CRMs): wheat flour (NIST 1567a), bovine liver (NIST 1577), peach leaves (NIST 1547), oyster tissue (NIST 1566b), and fish protein (DORM-3). The recovery values obtained for the CRMs were between 80 ± 4% (Cr) and 117 ± 5% (Cd), with relative standard deviations (RSDs) better than 5%, demonstrating that the proposed method offered good trueness and precision. Ten samples of pet food (five each of cat and dog food) were acquired at supermarkets in Aracaju city (Sergipe State, Brazil). Concentrations in the dog food ranged between 7.1 mg kg^{-1} (Ba) and 2.7 g kg^{-1} (Ca), while for cat food the values were between 3.7 mg kg^{-1} (Ba) and 3.0 g kg^{-1} (Ca). The concentrations of Ca, K, Mg, P, Cu, Fe, Mn, and Zn in the food were compared with the guidelines of the United States' Association of American Feed Control Officials (AAFCO) and the Brazilian Ministry of Agriculture, Livestock, and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento—MAPA).

1. Introduction

There has been worldwide growth in the numbers of domestic pets. The production of food for dogs and cats exceeded two million tons in 2010, constituting one of the fastest global growth areas [1,2]. In Brazil, the Ministry of Agriculture, Livestock, and Food Supply (MAPA) is responsible for regulating animal food [3]. The guidelines adopted are those established by the United States' Association of American Feed Control Officials (AAFCO) and the Food and Drug Administration (FDA) [4–6].

A wide variety of foods for dogs and cats are available on the market, including dry and wet products, canned foods, and treats, enabling owners choose a suitable product based on the race, size, and stage in life of the pet. The ingredients can include products and byproducts of animal origin, such as meat and bone meal, or plant-derived materials such as grains and flour, as well as minerals including phosphates. In addition to essential nutrients, these ingredients can contain contaminants including elements that are potentially toxic [2,5,7].

Alongside the increased pet ownership and feed production, there have been growing concerns regarding the safety of pet foods [3–6] and the possibility that chemical contaminants might be responsible for health damage to animals. Given the growing demand in this sector, studies have increasingly focused on the production and sale of reliable foodstuffs for animal nutrition [2,8,9].

The conventional procedure for the quantitative chemical analysis of solid samples is based on a preparation procedure employing digestion or extraction of the analytes, prior to instrumental measurements. The decomposition of biological

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materials and food samples using nitric acid, or a mixture of nitric acid and hydrogen peroxide, has been widely used. Improvements in sample preparation techniques have recently focused on the time involved, as well as simplicity and the ability to use dilute oxidants [10–13].

Dilute nitric acid is an attractive agent for the decomposition of organic matter, and has been widely applied to samples that have high carbon contents [14–18]. However, the efficiency of the digestion can be influenced by the existence of temperature gradients within the reaction vessel [13], and one way of improving performance is to supply pressurized oxygen in order to regenerate the nitric acid during the process [16,18]. In order to prevent safety issues involved in the use of pressurized oxygen in laboratories, an easier and more practical alternative is to supply oxygen to the reaction chamber in the form of hydrogen peroxide [19]. This procedure has been shown to be effective for the digestion of certified reference materials (CRMs) including apple tree leaves, bovine liver, and milk powder [16,19].

The determination of metals, metalloids, and non-metals in high carbon content samples can be performed using techniques that enable simultaneous multielement analysis, such as optical emission spectrometry (OES) or mass spectrometry (MS), both employing an inductively coupled plasma (ICP) for analyte atomization [20–23].

Experimental design methodology can be used to reduce analysis times and improve detection of analytes, identifying the individual variables that have greatest effects, as well as considering interactions between variables that influence the analytical response. The goal is to optimize the experimental conditions for best results, maximizing performance while reducing costs and analysis times [10–12,24].

Factorial design is a chemometric tool that can be used to optimize the parameters involved in chemical analysis, including the digestion procedure and other operational variables [12,21,25]. Costa et al. used factorial design to optimize a microwave-assisted acid digestion procedure for the degradation of bean samples prior to determination of Ca, Fe, Mg, Mn, and Zn [26]. Santos et al. employed response surface methodology with Box Behnken design to optimize the extraction of Ba, Ca, Cu, Fe, K, Mg, Mn, Sr, and Zn from beans, using ultrasonication, with subsequent analysis by multielement ICP OES [25]. In another work, Trevizan et al. used central composite design to evaluate the plasma conditions and to compare two liquid sample introduction systems for ICP OES aiming the determination of various elements in a non-fat milk powder material, while Guimarães-Silva et al. focused on the optimization of the ICP OES parameters addressing better emission signals of rare earths employing Doehlert design. [27,28] Kadar et al. used the same chemometric tool to optimize the ICP-MS collision/reaction cell conditions for the determination of elements likely to be interfered (V, Cr, Fe, Co, Ni, As and Se) in foodstuffs [29].

The present work interest is the optimization of an analytical method for determination of the mineral element content of dog and cat food by ICP OES, using experimental design techniques.

2. Experimental

2.1. Reagents and standard solutions

All reagents used were analytical grade, and solutions were prepared using ultrapure water (18.2 MΩ cm resistivity) obtained from a Milli-Q purification system (Millipore, USA). The nitric acid and hydrogen peroxide used were of Suprapure quality (Merck, USA). All material and glassware used in the experiments was previously decontaminated with a nitric acid solution (10% v/v) for 24 h [16], rinsed with ultrapure water, and dried at room temperature. The preparation of the external calibration curves employed Specsol® multielement standard solutions at concentrations of 100 mg L^{-1} (for Ag, Al, B, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Si, Sn, Sr, Ti, V, and Zn) or 1000 mg L^{-1} for elements present at higher concentrations in some of the samples, Ca, K, Mg, Al, Fe, Na, P, and S, which required an extended calibration curve. For carbon the stock solution was 10,000 mg L^{-1}. External Calibration curves were prepared in a range of concentrations from 0.1 to 5.0 mg L^{-1} for Cu, Sr, Zn, Mn, Cd, Ba and Cr and from 5.0 to 200.0 mg L^{-1} for Ca, S, Al, P, Mg and K. For the determination of residual carbon external calibration curve from 500 to 5000 mg L^{-1} was prepared.

2.2. Sample collection, storage, and moisture content determination

Ten pet food samples (5 for dogs and 5 for cats) were obtained at supermarkets in the city of Aracaju (Sergipe State, Brazil). In the laboratory, these were divided into 50 g portions and triturated in order to ensure homogeneity [11]. They were then stored away from light in polyethylene containers. The humidity content was determined gravimetrically, by weighing approximately 1 g of sample before and after lyophilization. The values of the concentrations obtained for the samples after analyses were corrected take into account the content of humidity measured by the gravimetric analysis.

2.3. Preparation of the dog and cat food samples

Approximately 0.25 g (dry weight) portions of sample were weighed out [30] and placed into microwaveable polytetrafluoroethylene tubes, in which 1.4 mL of HNO_3 (65% m/v) and 2.0 mL of H_2O_2 (30% m/v) were added. The mixture was allowed to rest for 30 min and then the volume was completed to 10 mL with ultrapure water [13]. The PTFE tubes were placed in a microwave oven and submitted to a two-stage heating program. In the first stage, the temperature was increased linearly to 180 °C in a 5 min interval at a maximum power of 400 W, and kept for 15 min. In the second stage, the temperature was maintained at 180 °C, and the power was increased to 800 W in 5 min, with the final conditions kept for 5 min. After this procedure, the samples were transferred to polyethylene tubes, and the volumes were made up to 15 mL with ultrapure water. The procedure was performed in triplicate [31]. To evaluate the quality of the reagents and the trueness of the analytical method blank solutions were prepared and certified reference materials were submitted to the same procedure. [32].

2.4. Quality control

The trueness and precision [15,33] of the proposed analytical method were evaluated by analysis of five CRMs, namely the National Institute of Standards and Technology (NIST) materials wheat flour (NIST 1567a), bovine liver (NIST 1577), oyster tissue (NIST 1566b), and peach tree leaf (NIST 1547), and the National Research Council Canada (NRC) material fish protein (DORM-3) [11,16–18,34,35]. These CRMs were selected based on their similarities to the ingredients used in the dog and cat foods.

2.5. Instrumentation and equipment

The samples were decomposed by microwave-assisted digestion using a MARSXPress microwave oven (CEM, USA), equipped with PTFE flasks and sensors to monitor pressure and temperature inside the reaction vessels. Analysis of the sample after digestion employed a Vista Pro (Varian, Australia) optical emission spectrometer with inductively coupled plasma and axial

Table 1
Operational conditions of the ICP OES with an axial configuration.

Parameter	Characteristics	
Radiofrequency power (W)	1200	
Plasma gas flow rate (L min^{-1})	15.0	
Auxiliary gas flow rate (L min^{-1})	1.5	
Sample uptake rate (mL min^{-1})	0.8	
Nebulizer gas flow rate (L min^{-1})	1.0	
Nebulizer type	Concentric, sea spray	
Spray chamber	Cyclone type	
Replicates	3	
Injector tube diameter (mm)	2.4	
Signal integration time (s)	1.0	
Wavelength (nm)	Ca I (373.690)	S I (181.972)
	C I (193.027)	Cu I (324.754)
	Al I (309.271)	P I (178.222)
	Mg I (285.213)	Sr II (407.771)
	Zn I (213.856)	Mn II (260.569)
	Cd I (226.502)	Fe II (238.204)
	K I (766.491) Cr (267.716)	Ba II (233.527)

I—Atomic line.
II—Ionic line.

viewing configuration. The operating conditions of the instrument are shown in Table 1.

2.6. Optimization strategy

The analytical procedure of microwave-assisted digestion was optimized using a full two-level factorial design. The factors were the concentrations of the two reagents diluted, hydrogen peroxide and nitric acid. The efficiency of the organic matter decomposition (EOMD, expressed as %) was used as the response of the factorial design. A three-level factorial design with two variables (RF power and nebulization gas flow rate) was employed to optimize the ICP OES operational conditions. The response was the ratio between the intensities of the magnesium emission lines, Mg II (280.265 nm)/ Mg I (285.208 nm). Triplicates of the central point were performed to evaluate experimental error. Experimental data were processed using the Statistica 6.0 computer program.

3. Results and discussion

3.1. Optimization of sample digestion conditions

Sample preparation is a critical step in the analytical process, especially in the case of foods, since the high organic matter content of the matrix makes it necessary to use mineral acids and hydrogen peroxide for digestion [11,18,36]. An effective alternative procedure for the decomposition of organic matter is to use diluted reagents and microwave-assisted digestion [13–19,37].

A two-level factorial design was used to identify the best conditions for digestion of the pet food samples, employing diluted nitric acid and hydrogen peroxide as an auxiliary reagent, with the aim of minimizing the amount of reagents required. Approximately 0.25 g portions of commercial dog food were used in these experiments. Table 2 shows the 2^2 factorial design matrix with three central points used to evaluate the best ratio between the diluted reagents (HNO_3 and H_2O_2). The efficiency of the organic matter decomposition (EOMD, expressed as % m/m) was used as the response for the factorial design.

Starting with the residual carbon content (RCC) obtained in each experiment, the efficiency of sample digestion was calculated using the expression $EOMD = [(TCC - RCC)/TCC] \times 100$, where TCC is the total carbon content. The residual carbon

Table 2
Matrix of the 2^2 full factorial design with three central points.

Experiment	Values		EOMD (% m/m)
	HNO_3 (mol L^{-1})	H_2O_2 (% m/v)	
1	(−1)/2.0	(−1)/3.0	84.4
2	(+1)/5.0	(−1)/3.0	84.2
3	(−1)/2.0	(+1)/6.0	87.6
4	(+1)/5.0	(+1)/6.0	87.4
5	(0)/3.5	(0)/4.5	87.3
6	(0)/3.5	(0)/4.5	86.6
7	(0)/3.5	(0)/4.5	86.1

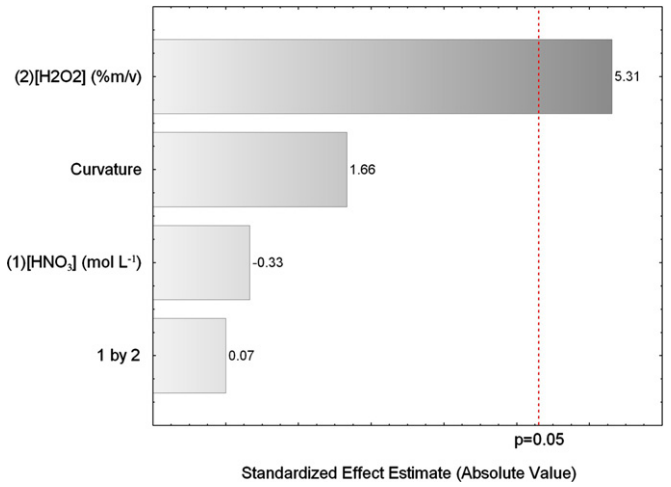

Fig. 1. Pareto chart for the 2^2 factorial design (effects of the concentrations of HNO_3 and H_2O_2).

concentration was determined by ICP OES using the carbon emission line at 193.027 nm [13,27].

The results obtained for the principal effects and interactions are illustrated in the Pareto chart shown in Fig. 1. The use of the 2^2 factorial design revealed that the only variable which significantly influenced the digestion of the dog food sample was the H_2O_2 concentration, as reflected in EOMD. An increase in the concentration of the auxiliary oxidant in the medium led to greater EOMD values, due to regeneration of the nitric acid during the digestion process [19]. No statistically significant effects (at the 95% confidence level) were observed for the HNO_3 concentration, the interactions between the variables, or the curvature estimate (indicating an absence of curvature in the linear model). Therefore, the conditions established for digestion of the dog food sample were the same as those used in the third experiment (EOMD = 87.6%), the addition of 2 mol L^{-1} HNO_3 and 6% v/v H_2O_2 to a final volume of 10 mL, followed by microwave-assisted digestion.

3.2. Optimization of the ICP OES operating conditions

According to Mermet [38], the RF power and the residence time (determined by the gas flow rate and the diameter of the injector) are the most critical parameters affecting the plasma conditions, as demonstrated by the ratio of the intensities of the Mg II (280.265 nm) and Mg I (285.208 nm) emission lines. The argon gas rate to reach the exit of the injector tube depends on the nebulizer gas flow rate and the internal diameter of the injector, while the quantity of aerosol depends only on the nebulizer gas flow rate. The magnesium emission intensity is greater at higher nebulizer gas flow and RF power.

A three-level factorial design with two variables (RF power and nebulization gas flow rate) was therefore employed to optimize the operational conditions of the ICP OES so as to reduce costs and minimize the wear of the equipment and, at the same time, to provide greater precision and trueness during multielement analyses. The response was the ratio between the intensities of the magnesium emission lines, Mg II (280.265 nm)/Mg I (285.208 nm), measured using two different solutions [38]. The first was an extract of commercial dog food, containing Mg at a concentration of 10 mg L^{-1}, approximately. The second was a standard solution prepared with 10 mg L^{-1} of Mg, with the addition of 0.2 mg L^{-1} of Ag, Al, B, Co, Cr, Cu, Mn, Ni, Pb, Si, Sn, Sr, Ti, V, Zn, As, Mo, Li, Cd, Ba, and Hg, plus 2 mg L^{-1} of Ca, Fe, K, Mg, and P, in order to simulate a dog food extract.

An Mg II/Mg I emission intensity ratio equal to or greater than 8, point to a robust plasma condition, wherein the ICP system able to accommodate alterations in the concentrations of major elements, acids, and other components, without any significant variation in the intensities of the analyte lines [18,19,38].

Table 3 shows the three-level factorial design matrix for two variables used to optimize the operational conditions of the ICP OES. The instrumental parameters are achieved with the responses (Mg II/Mg I emission intensity ratios) obtained for the digested dog food sample and the synthetic standard solution, and also with the multiple response as function of the Mg II/Mg I emission intensity ratios for the two different solutions.

Three replications of the central point were carried out in order to estimate the experimental error, and all experiments were performed in random order. The final assessment was made using the multiple response (MR) desirability function approach [19,29,39]. For MR calculation the following expression was used:

$$MR = \left(\frac{(MgII/MgI)}{(MgII/MgI)max}\right)_{digest} + \left(\frac{(MgII/MgI)}{(MgII/MgI)max}\right)_{std},$$

where, Mg II/Mg I is the ratio value between the intensities of the Mg II (280.265 nm) and Mg I (285.208 nm) emission lines, and (Mg II/Mg I) max is the maximum value of the ratio between the intensities of the emission lines found in the experiments, for both the digested dog food sample and the standard solution prepared as described previously.

The three-level factorial design for two variables, with three central points, aims to investigate the best fit to a mathematical model (linear or quadratic), using response surface methodology to generate a multiple response given by a mathematical function [25,28,40,41]. As shown in the Pareto chart in Fig. 2, the influence of the nebulizer gas flow rate was 4.5 times greater than that of the linear RF power [19]. The interaction between the two variables was also significant (at a confidence level of 95%), with a positive value of 4.76, confirming the existence of a positive relationship between the variables. According to both, Guimaraes et al. and Trevisan et al., the RF power and nebulizer gas flow are the significant variables for robust plasma. [27,28].

After adjusting the quadratic model to the data obtained from experiments using three-level factorial design for two variables, analysis of variance (ANOVA) was employed to evaluate the importance of each variable. The summary of ANOVA is shown in Table 4. The quadratic model was significant ($F_{calculated;2.9}$= 24.58; $F_{table;2.9}$=4.26; *p-value*=2.26E-4) do not showing lack of fit ($F_{calculated;3.3}$=1.9; $F_{table;3.3}$=9.3; *p-value*=3.1E−1) for a level of 95%, using the multiple response function. The two variables and their interactions had a significant effect on the ratio of the Mg I and Mg II emission intensities [22,23,30,31,42–44], except for RF power quadratic fit; ($F_{calculated;1.3}$=7.6; $F_{table;1.3}$=10.1; *p*-value=7.0E−2), for which no obvious analytical explanation was found. Other evidence for the fit of the experimental data to the quadratic model was shown by the good correlation (r=0.999) found between the experimental and predicted values [41]. In summary, increase in both, sample nebulization rate and RF power, improved the performance of the system.

The experimental design results indicate that the maximum response occurred beyond the boundaries of the parameter values employed. The Mg II/Mg I emission intensity ratio increased up to the maximum nebulizer gas flow rate used, and at higher RF power, which enhanced the plasma robustness. However, the use of high RF power accelerates the wear of the system, causing cost implications [29]. The best compromise conditions for operation of the ICP OES were therefore selected by visual inspection of the results of the three-level factorial design for two variables. These

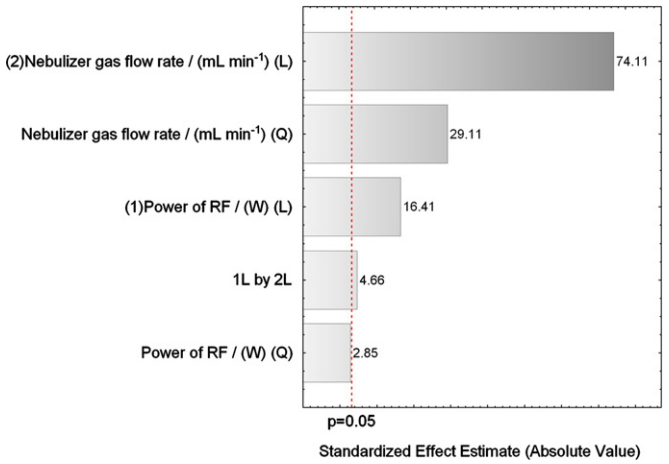

Fig. 2. Pareto chart for the three-level factorial design with three central points (effects of nebulizer gas flow rate and RF power).

Table 3
Three-level factorial design for two variables with three central points, used to optimize the ICP OES operating conditions.

Experiment	RF power (W)	Nebulizer gas flow rate (L min^{-1})	Mg II/Mg I ratio—standard solution	Mg II/Mg I ratio—digested sample solution	Multiple response
1	(−1)/1000	(−1)/0.6	1.63	1.52	0.29
2	(−1)/1000	(0)/0.8	7.53	6.92	1.31
3	(−1)/1000	(+1)/1.0	8.72	8.93	1.60
4	(0)/1200	(−1)/0.6	2.23	2.02	0.39
5	(0)/1200	(0)/0.8	8.95	8.28	1.57
6	(0)/1200	(+1)/1.0	9.96	10.29	1.84
7	(+1)/1400	(−1)/0.6	2.66	2.51	0.47
8	(+1)/1400	(0)/0.8	9.52	8.98	1.68
9	(+1)/1400	(+1)/1.0	10.84	11.19	2.00
10	(0)/1200	(0)/0.8	8.95	8.28	1.57
11	(0)/1200	(0)/0.8	8.54	8.17	1.52
12	(0)/1200	(0)/0.8	8.98	8.10	1.55

Table 4
Statistical analysis: ANOVA.

Source	Sum of squares	Degrees of freedom	Mean squares	*F*-value	*p*-value
(1)Power of RF (W) (L)	1.5E−1	1	1.5E−1	3E+2	4.9E−4
Power of RF (W) (Q)	3.9E−3	1	3.9E−3	7.6	1.0E−1
(2) Nebulizer gas flow rate (L min^{-1}) (L)	3.1E+1	1	3.1E+1	6.1E+3	5.0E−6
Nebulizer gas flow rate (L min^{-1}) (Q)	4.8E−1	1	4.8E−1	9.4E+4	1.0E−4
Interaction (1 L × 2 L)	1.1E−2	1	1.1E−2	2.3E+1	1.86E−2
Lack of fit	2.9E−3	3	9.6E−4	1.9	3.0E−1
Pure error	1.5E−3	3	5.1E−4		
Total SS	3.8E+1	11			

Table 5
BEC, LOD, and LOQ values obtained for analysis of dog and cat food samples by ICP OES.

Analytical parameter	Ca	P	S	Zn	Ba	K	Sr	Mn	Mg	Al	Cd	Cu	Fe	Cr
BEC (mg L^{-1})	0.37	0.16	0.30	0.02	0.001	0.09	0.0004	0.001	0.01	0.54	0.002	0.009	0.001	0.001
LOD (mg kg^{-1})	26	9	6	0.6	0.02	0.8	0.03	0.08	0.5	2.0	0.04	0.08	0.3	0.03
LOQ (mg kg^{-1})	87	31	18	2.0	0.08	2.7	0.09	0.3	1.8	7.0	0.12	0.27	0.9	0.1

values were an RF power of 1.2 kW and a nebulizer gas flow rate of 1.0 L min^{-1} [34]. A previous work showed that a nebulizer gas flow rate of 0.6 L min^{-1} and RF power of 1.2 kW are sufficient to provide robust plasma conditions and to ensure an Mg II/Mg I emission intensity ratio ≥ 8 [29].

3.3. Figures of merit

The performance of the proposed method was evaluated under the optimized conditions. The limits of detection (LOD) and quantification (LOQ) were calculated using the background equivalent concentration (BEC) and the signal-to-background ratio (SBR), according to IUPAC recommendations [45]: $BEC = C_{RS}/SBR$, being $SBR = (I_{RS} - I_{blank})/I_{blank}$, C_{RS} the reference element concentration in the solution, and I_{RS} and I_{blank} the emission intensities for the reference element and blank solutions, respectively. The precision was expressed as relative standard deviation (RSD), calculated using 10 consecutive measurements of the blank solution. The LOD was then calculated as $(3 \times RSD \times BEC)/100$, and the LOQ was calculated as $3.3 \times LOD$. The LOQ values ranged from 0.08 μg g^{-1} (Ba) to 87 μg g^{-1} (Ca), and were within the range considered acceptable for determination of Ca, P, S, Zn, Ba, K, Sr, Mn, Mg, Al, Cd, Cu, Cr, and Fe.The analytical parameters are summarized in Table 5.

The quality of the results obtained using the optimized method was confirmed by analysis of the five certified reference materials representative of the dog and cat food components, as listed above. The results are given in Table 6, expressed as the means ± 95% confidence intervals ($n=3$). The agreement with the certified values ranged from 80 ± 4% (Cr) to 117 ± 5% (Cd). The absolute relative errors between the values were from 0 to 20%, and the precision, expressed as the relative standard deviation (RSD), was better than 5% for all elements except Cr (> 12%). The values presented satisfactory trueness and precision of the proposed method [21]. The multielement determination of S, Al, Ca, Cu, K, Mg, Mn, P, Zn, Sr, Cd, Fe, Cr, and Ba in the five CRMs could be considered quantitative, since the absolute relative errors did not exceed ± 20% [36].

3.4. Application of the technique using samples of dog and cat foods

The optimized analytical method using microwave-assisted digestion and detection by ICP OES in the analysis of dry dog and cat foods was applied. Ten samples of different brands were used (five dog foods and five cat foods). The results of the determinations of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, P, S, Sr, and Zn are shown in Table 7 for the dog foods and in Table 8 for the cat foods. The results are expressed as means ± the 95% confidence intervals ($n=3$).

According to the Brazilian Ministry of Agriculture, Livestock, and Food Supply (MAPA), the humidity content of dry dog and cat food must not exceed 12%. Here, the measured values were 8–10% (dog food) and 7–9% (cat food), showing that the foods complied with current legislation [3].

For the elements Cd (< 0.12 mg kg^{-1}) and Cr (< 0.1 mg kg^{-1}), the concentrations in the samples were below the quantification limits of the proposed method. In other work, Duran et al. [9] found concentrations of between 0.60 and 2.47 mg kg^{-1} (Cd), and between 0.58 and 3.73 mg kg^{-1} (Cr), in samples of dog and cat foods marketed in Turkey.

Calcium, phosphorus, potassium, magnesium, and sulfur were present at high concentrations in the samples, since these elements are important animal nutrients. In dog food, the ranges of measured values were 1.6 ± 0.2–2.7 ± 0.3 g kg^{-1} (Ca), 2.2 ± 0.1–2.4 ± 0.2 g kg^{-1} (P), 1.00 ± 0.02–1.3 ± 0.2 g kg^{-1} (K), 0.14 ± 0.01–0.25 ± 0.01 g kg^{-1} (Mg), and 0.36 ± 0.03–0.78 ± 0.08 g kg^{-1} (S). In cat food, the values were 0.79 ± 0.10–3.0 ± 0.2 g kg^{-1} (Ca), 1.3 ± 0.1–2.7 ± 0.2 g kg^{-1} (P), 0.92 ± 0.1–1.3 ± 0.1 g kg^{-1} (K), 0.11 ± 0.01–0.34 ± 0.05 g kg^{-1} (Mg), and 0.36 ± 0.1–0.57 ± 0.04 g kg^{-1} (S).

For the micronutrients, in dog food the ranges of concentrations were 147 ± 7–606 ± 13 mg kg^{-1} (Fe), 15.5 ± 1.7–34.1 ± 3.2 mg kg^{-1} (Cu), 6.5 ± 1.0–149 ± 12 mg kg^{-1} (Mn), 106 ± 5–419 ± 5 mg kg^{-1} (Zn), 7.1 ± 0.8–25.1 ± 1.7 mg kg^{-1} (Ba), 20.3 ± 2.1–22.4 ± 2.4 mg kg^{-1} (Sr), and 45.5 ± 6.4–2835 ± 8 mg kg^{-1} (Al). In cat food, the ranges were 129 ± 3–569 ± 4 mg kg^{-1} (Fe), 6.4 ± 0.5–29.5 ± 2.6 mg kg^{-1} (Cu), 7.8 ± 0.02–70.1 ± 10 mg kg^{-1} (Mn), 60.8 ± 2.5–235 ± 5.3 mg kg^{-1} (Zn), 3.7 ± 0.5–29.0 ± 3.2 mg kg^{-1} (Ba), 10.0 ± 1.0–39.5 ± 4.7 mg kg^{-1} (Sr), and 33.3 ± 8.4–475 ± 8 mg kg^{-1} (Al).

The concentrations of the major nutrient elements Ca, K, Mg, and P were below the thresholds established by AAFCO [4,6]. The P concentration was in accordance with Brazilian legislation [3], for both types of food.

4. Conclusions

Dog and cat foods were prepared for analysis by microwave-assisted digestion using dilute solutions of nitric acid and hydrogen peroxide. The proportions of the reactants used in the digestion were selected using a 2^2 two-level full factorial design

Table 6
Results of the analysis of certified reference materials by ICP OES.

		S/%	Al/ mg kg^{-1}	Ca/%	Cu/mg kg^{-1}	K/%	Mg/%	Mn/ mg kg^{-1}	P/%	Zn/ mg kg^{-1}	Sr/ mg kg^{-1}	Cd/mg kg^{-1}	Fe/mg kg^{-1}	Ba/ mg kg^{-1}	Cr/ mg kg^{-1}
NIST 1567a	**Measured value**	0.168 ± 0.003	5.9 ± 1.4	0.0192 ± 0.0008	2.2 ± 0.1	0.1260 ± 0.0005	0.0370 ± 0.0004	8.8 ± 0.05	0.139 ± 0.005	11.0 ± 0.7					
	Certified value	0.165 ± 0.002	5.7 ± 1.3	0.0191 ± 0.0004	2.1 ± 0.2	0.133 ± 0.003	0.040 ± 0.002	9.4 ± 0.9	0.134 ± 0.006	11.6 ± 0.4					
	Recovery (%)	102 ± 2	104 ± 24	100 ± 4	103 ± 4	95.0 ± 0.3	94 ± 1	94 ± 0.5	103 ± 4	95 ± 6					
	Relative error (%)	−2 ± 2	−4 ± 4	1 ± 4	5 ± 4	−5 ± 0.3	−8 ± 1	−6 ± 0.5	4 ± 4	−5 ± 6					
NIST 1577	**Measured value**			104 ± 17[a]	188 ± 3	0.93 ± 0.02	607 ± 6 [a]	10.4 ± 0.16	1.10 ± 0.02	137 ± 2	0.13 ± 0.01	0.31 ± 0.01	267 ± 2		
	Certified value			124 ± 6 [a]	193 ± 10	0.97 ± 0.06	604 ± 9 [a]	10.3 ± 1.0	(1.1)	130 ± 13	(0.14)	0.27 ± 0.04	268 ± 8		
	Recovery (%)			84 ± 14	98 ± ± 2	96 ± 2	101 ± 1	101 ± 2	98 ± 2	105 ± 1	90 ± 8	117 ± 5	100 ± 1		
	Relative error (%)			−16 ± 14	−2 ± 2	−4 ± 2	0 ± 1	−2 ± 2	0 ± 2	5 ± 1	−7 ± 8	15 ± 5	0 ± 1		
NIST 1547	**Measured value**	0.20 ± 0.01	244 ± 9	1.36 ± 0.04	3.6 ± 0.2	2.28 ± 0.11	0.372 ± 0.008	84 ± 2	0.128 ± 0.006	16.7 ± 0.3	51 ± 1		184 ± 3	106 ± 2	
	Certified value	(0.2)	249 ± 8	1.56 ± 0.02	3.7 ± 0.4	2.43 ± 0.03	0.432 ± 0.008	98 ± 3	0.137 ± 0.007	17.9 ± 0.4	53 ± 4		218 ± 14	124 ± 4	
	Recovery (%)	84 ± 4	98 ± 4	87 ± 3	98 ± 4	94 ± 5	86 ± 2	86 ± 2	94 ± 4	93 ± 2	97 ± 2		84 ± 1	86 ± 2	
	Relative error (%)	0 ± 4	−2 ± 4	−13 ± 3	−3 ± 4	−6 ± 5	−14 ± 2	−14 ± 2	−7 ± 4	−7 ± 2	−4 ± 2		−16 ± 1	−15 ± 2	
DORM − 3	**Measured value**		1872 ± 98		15.9 ± 1.18					51.3 ± 3.7		0.247 ± 0.043	322 ± 29		1.52 ± 0.07
	Certified value		(1700)		15.5 ± 0.63					51.3 ± 3.1		0.290 ± 0.020	347 ± 20		1.89 ± 0.17
	Recovery (%)		110 ± 6		102 ± 8					100 ± 7		85 ± 15	93 ± 8		80 ± 4
	Relative error (%)		10 ± 6		3 ± 8					0 ± 7		−15 ± 15	−7 ± 8		−20 ± 4
NIST 1566b	**Measured value**	0.6137 ± 0.0248		0.0791 ± 0.0028	71.1 ± 2.6	0.624 ± 0.027	0.1024 ± 0.0048	17.5 ± 0.6		1458 ± 61	6.4 ± 0.4	2.46 ± 0.09	187.9 ± 11.2		
	Certified value	0.6887 ± 0.0140		0.0838 ± 0.0020	71.6 ± 1.6	0.652 ± 0.009	0.1085 ± 0.0023	18.5 ± 0.2		1424 ± 46	6.8 ± 0.2	2.48 ± 0.08	205.8 ± 6.8		
	Recovery (%)	89 ± 4		94 ± 3	99 ± 4	96 ± 4	94 ± 4	95 ± 3		102 ± 4	93 ± 6	99 ± 4	91 ± 5		
	Relative error (%)	−11 ± 4		−6 ± 3	−1 ± 4	−4 ± 4	−6 ± 4	−5 ± 3		2 ± 4	−6 ± 6	−1 ± 4	−9 ± 5		

NIST 1567a—CRM of wheat flour; NIST 1547—CRM of peach leaves; NIST 1577—CRM of bovine liver; NIST 1566b—CRM of oyster tissue; DORM-3—CRM of fish protein; ( )—non-certified value; recovery (%)=[(measured value − certified value)/certified value] × 100; Relative error (%)=[(measured value–certified value)/certified value] × 100; analysis in triplicate (n=3); results expressed as average concentration ± confidence interval (95% level).

[a] Concentration in mg kg^{-1}.

Table 7
Concentrations of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, P, S, Sr, and Zn determined in dog foods by ICP OES, and minimum and maximum concentrations permitted by regulatory agencies.

Food	Moisture (%)	Flavor	Al/mg kg^{-1}	Ba/mg kg^{-1}	Ca/g kg^{-1}	Cu/mg kg^{-1}	Fe/mg kg^{-1}	K/g kg^{-1}	Mg/g kg^{-1}	Mn/mg kg^{-1}	P/g kg^{-1}	S/g kg^{-1}	Sr/mg kg^{-1}	Zn/mg kg^{-1}
Dog food (1)	10	Meat and bones	236 ± 6	25.1 ± 1.7	2.3 ± 0.2	15.5 ± 1.7	606 ± 13	1.3 ± 0.2	0.33 ± 0.03	92.3 ± 3.6	2.4 ± 0.2	0.36 ± 0.03	20,3 ± 2.1	251 ± 3
Dog food (2)	8	Meat and vegetables	2835 ± 8	24.6 ± 3.7	2.5 ± 0.1	26.5 ± 0.4	421 ± 13	1.2 ± 0.05	0.23 ± 0.01	13.7 ± 4.3	2.2 ± 0.1	0.52 ± 0.004	21,1 ± 1.2	106 ± 5
Dog food (3)	9	Meat	45.5 ± 6.4	24.2 ± 1.1	2.4 ± 0.3	17.4 ± 0.3	337 ± 7	1.0 ± 0.02	0.25 ± 0.01	93.2 ± 4.6	2.2 ± 0.2	0.38 ± 0.007	21,4 ± 2.0	293 ± 4
Dog food (4)	8	Meat and vegetables	475 ± 10	21.5 ± 4.0	2.7 ± 0.3	22.2 ± 0.8	147 ± 7	1.0 ± 0.1	0.14 ± 0.01	6.5 ± 1.0	2.2 ± 0.2	0.43 ± 0.03	21,4 ± 2.7	324 ± 3
Dog food (5)	10	Cereals	54.0 ± 5.7	7.1 ± 0.8	1.6 ± 0.2	34.1 ± 3.2	595 ± 8	1.1 ± 0.2	0.16 ± 0.02	149 ± 12	2.3 ± 0.2	0.78 ± 0.08	22,4 ± 2.4	419 ± 4
Minimum allowable concentration			***	***	6.0[a]	7.3[a]	80.0[a]	6.0[a]	0.4[a]	5.0[a]	5.0[a] 0.6[b]	***	***	120[a]
Maximum allowable concentration			***	***	25.0[a] 2.4[B]	250[a]	3000[a]	***	3.0[a]	***	16.0[a]	***	***	1000[a]

[a] Values established by AAFCO.
[b] Values established by MAPA.

Table 8
Concentrations of Al, Ba, Ca, Cu, Fe, K, Mg, Mn, P, S, Sr, and Zn determined in cat foods by ICP OES, and minimum and maximum concentrations permitted by regulatory agencies.

Food	Moisture (%)	Flavor	Al/mg kg^{-1}	Ba/mg kg^{-1}	Ca/g kg^{-1}	Cu/mg kg^{-1}	Fe/mg kg^{-1}	K/g kg^{-1}	Mg/g kg^{-1}	Mn/mg kg^{-1}	P/g kg^{-1}	S/g kg^{-1}	Sr/mg kg^{-1}	Zn/mg kg^{-1}
Cat food (1)	9	Mixed	475 ± 8	24.6 ± 2.8	2.8 ± 0.3	24.7 ± 0.3	569 ± 4	1.1 ± 0.05	0.24 ± 0.01	43.7 ± 6.2	2.5 ± 0.3	0.52 ± 0.03	39,5 ± 4.7	165 ± 6
Cat food (2)	8	Meat	63.1 ± 7.8	3.7 ± 0.5	0.79 ± 0.10	17.9 ± 1.0	129 ± 3	1.3 ± 0.1	0.11 ± 0.01	7.8 ± 0.02	1.3 ± 0.1	0.57 ± 0.04	10,0 ± 1.0	235 ± 5
Cat food (3)	8	Meat, rice, and corn	47.6 ± 3.8	17.5 ± 1.7	2.0 ± 0.2	29.5 ± 2.6	325 ± 4	1.1 ± 0.1	0.20 ± 0.02	28.0 ± 5.4	2.0 ± 0.1	0.40 ± 0.04	37,6 ± 3.7	208 ± 3
Cat food (4)	7	Mixed	78.7 ± 2.9	15.2 ± 0.4	2.0 ± 0.4	6.4 ± 0.5	201 ± 8	0.92 ± 0.1	0.19 ± 0.02	13.7 ± 5.1	1.8 ± 0.2	0.43 ± 0.02	29,5 ± 3.1	60,8 ± 22
Cat food (5)	8	Fish	33.3 ± 8.4	29.0 ± 3.2	3.0 ± 0.2	18.1 ± 2.1	184 ± 10	1.3 ± 0.2	0.34 ± 0.05	70.1 ± 10	2.7 ± 0.2	0.36 ± 0.1	36,6 ± 2.0	110 ± 6
Minimum allowable concentration			***	***	6.0[a]	5.0[a]	80.0[a]	6.0[a]	0.4[a]	7.5[a]	5.0[a] 0.6[b]	***	***	75.0[a]
Maximum allowable concentration			***	***	2.4[b]	***	***	***	***	***	***	***	***	2000[a]

[a] Values established by AAFCO.
[b] Values established by MAPA.

with three central points. The analyses were performed by ICP OES in axially viewed configuration. The instrumental conditions were optimized by a three-level factorial design for two variables with three central points, resulting in the use of an RF power of 1.2 kW and a nebulizer gas flow rate of 1.0 L^{-1} min^{-1}.

The trueness of the analytical method was confirmed by analysis of certified reference materials consisting of wheat flour, peach leaves, bovine liver, fish protein, and oyster tissue. The measured concentrations were in good agreement with the certified values for S, Al, Ca, Cu, K, Mg, Mn, P, Zn, Sr, Cd, Fe, Ba, and Cr, varying between $80 \pm 4\%$ (Cr) and $117 \pm 5\%$ (Cd) of the certified values. The precision, expressed as RSD, was better than 5% (with the exception of Cr). The optimized method was satisfactorily applied to quantitative multielement determination of the analytes in dog and cat food samples.

Evaluation of the elemental composition of commercial dog and cat foods showed that the macronutrients Ca, K, Mg, and P were present at levels below the minimum established by AAFCO. On the other hand, levels of the micronutrients Cu, Fe, Mn, and Zn were in accordance with the AAFCO values. It should be noted that the values established by AAFCO [4,6] are higher than those established by Brazilian legislation [3] and more elements are considered for dog food than for cat food. The concentrations of the essential micronutrients Cu, Fe, Mn, and Zn in the dog and cat foods were consistent with the limits established by AAFCO, except for Zn in dog food2, where the value was below the minimum allowable concentration [4,6]. The elements Al, Ba, Cd, S, and Sr are not included in legislation concerning of dog and cat foods [3,6,9].

The humidity contents in the ten samples of dry dog and cat food were within the specified Brazilian legal limits.

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