Brazilian Journal of Analytical Chemistry
2024, Volume 11, Issue 42, pp 41-54
doi: 10.30744/brjac.2179-3425.AR-4-2023

BrJAC

ARTICLE

Closed-Vessel Conductively Heated Digestion of Dry Dog Food for Spectrometric Determination of Essential Nutrients

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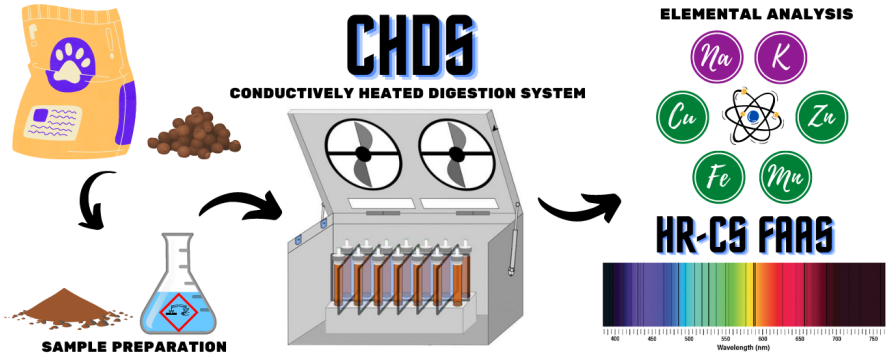

The closed-vessel conductively heated digestion system (CHDS) was evaluated to digest dry dog foods for further determination of K, Na, Cu, Fe, Mn, and Zn by high-resolution continuum source flame atomic absorption spectrometry (HR-CS FAAS). The CHDS method was optimized using a fractional factorial design with five variables (HNO_3 concentration, H_2O_2 volume, temperature, holding time, and pre-digestion time) at two levels. The accuracy of the CHDS procedure was checked by the analysis of reference materials from the National Institute of Standards and Technology (NIST SRM 1577b Bovine Liver and 2976 Mussel Tissue) and Brazilian Agricultural Research Corporation (Embrapa MR-E1002A Fish Food). Also, the digestion efficiencies were calculated from residual carbon contents (RCCs). The RCC and blank values in the CHDS digested samples were consistently low, which is suitable for determinations using ICP OES and ICP-MS techniques. For comparison, all samples were also digested by microwave-assisted digestion in closed vessels (MW-AD). Results for Na, K, Cu, Fe, Mn, and Zn determined in sample digests obtained by CHDS were not statistically different at a 95% confidence level from those observed for MW-AD. Limits of quantification (LOQ) calculated from digests in CHDS and MW-AD were comparable, and the values provided adequate limits for elemental determinations in dog foods. Data from mineral composition and moisture were employed in a clustering analysis (HCA) and the discrimination of the samples among different manufacturers and food for dogs at different life stages was possible.

Keywords: dog food, sample preparation, CHDS, HR-CS FAAS, pet nutrition

Cite: Costa, R. C. V.; Santiago, J. V. B.; Ferreira, E. C.; Virgilio, A.; Gomes Neto, J. A. Closed-Vessel Conductively Heated Digestion of Dry Dog Food for Spectrometric Determination of Essential Nutrients. *Braz. J. Anal. Chem.* 2024, *11* (42), pp 41-54. http://dx.doi.org/10.30744/brjac.2179-3425.AR-4-2023

Submitted 10 January 2023, Resubmitted 07 March 2023, 2nd time Resubmitted 20 April 2023, Accepted 30 April 2023, Available online 02 June 2023.

INTRODUCTION

The pet industry has been experiencing substantial growth, with the global pet care market valuation around US$ 138 - 179 billion in 2020 and expected to reach US$ 240 - 270 billion by 2030.[1,2] The pet food segment stands for 70% of those values, and pet owners have been increasing their concerning about providing appropriate nutrition and healthier diets to their animals,[3] considering the quality of ingredients as the main characteristic to be taken into consideration for the food selection.[4] Considering the high demand, large-scale production of dry pet foods under controlled conditions requires manufacturers to implement good practices and strict quality control.[3] Macronutrients such as Na and K are essential in a dog's acid-base balance, regulation of osmotic pressure, and nerve impulse generation and transmission. Micronutrients are also vital in processes as the synthesis of blood components and energy metabolism (Fe); defense against oxidative damage and formation of connective tissue, blood cells, melanin, and myelin (Cu); enzyme functions, bone development and neurological function (Mn); and enzyme reactions, cell replication, protein and carbohydrate metabolism, skin function and wound healing (Zn).[5] Whereas the supplementation of essential minerals in dry pet food is crucial for health maintenance, the inorganic element analysis takes an important role in food safety and quality of both raw materials and commercial products formulated to achieve diet requirements for specific breeds and life stages.[5,6]

Most spectrometric techniques for inorganic elemental analysis rely on sample introduction systems basically fit for aqueous solutions. Thus, the solid samples must be chemically digested by dry or wet decomposition methods.[7] Wet digestions using liquid reagents in closed vessels are frequently employed in most spectroscopy methods.[8] Microwave-assisted digestion systems (MW-AD) using closed vessels are well-established, robust, and highly efficient, but the acquisition and maintenance of equipment are costly, which impairs MW-AD implementation in most small to medium-sized laboratories.[9]

The conductively heated digestion system (CHDS) is a simple and low-cost combination of closed vessels and conductively heating in digester blocks. This sample preparation technique has been applied for raw meats, milk, chocolate, coffee, biomass (sugarcane, eucalyptus, banana), vegetables, biochar, oyster shell flour, bone meal, swine manure, aiming at the elemental determinations by atomic absorption and plasma-based spectrometry.[10-13]

The elemental composition of dry dog foods obtained by chemical analysis may be a useful input to perform classifications by means of hierarchical cluster analysis (HCA), which is scarcely explored in the literature.[14] Also, the multivariate optimization and the digestion of dry dog food by CHDS have not yet been evaluated. The present work aims to evaluate the CHDS for digestion of dry dog foods (from distinct manufacturers and intended for different stages of pet development) for determination of K, Na, Cu, Fe, Mn, and Zn by HR-CS FAAS. The accuracy was assessed by analysis of bovine liver, mussel tissue and fish food reference materials. For comparative purposes, all dry dog foods were also analyzed after digestion by MW-AD.

MATERIALS AND METHODS

Instrumentation

All dry dog food samples and certified reference materials were digested using a conductively-heated digestion system (CHDS) composed of a 28-slot heating block, a temperature control terminal and two cooling fans built-in an acid-resisting cabinet with an acrylic safety shield. Further details on the CHDS instrumental setup can be found elsewhere.[11] A Multiwave microwave-assisted sample preparation system (Anton Paar, Graz, Austria), equipped with a 6-position rotor for 50 mL quartz vessels was used for comparison. The digestion efficiencies were evaluated by the quantification of the residual carbon content (RCC) using a TOC-L CSH/CSN (Shimadzu, Kyoto, Japan) carbon analyzer system.

The elemental determinations were performed in a contrAA® 300 high-resolution continuum source flame atomic absorption spectrometer (Analytik Jena AG, Jena, TH, Germany) equipped with a 300 W Xenon short-arc lamp XBO 301 (GLE, Berlin, BE, Germany) operating in a hot-spot mode as a continuum radiation source, a compact high-resolution double-Echelle grating monochromator (with a spectral

bandwidth < 2 pm per pixel in the far ultraviolet range) and a charge-coupled device (CCD) array detector.[15] All measurements were performed in three repetitions using an injection module (SFS 6), with a load time of 5.0 s, injection time of 10 seconds and the aspiration rate was maintained at 5.0 mL min^{-1}. The spectrometer operating conditions were automatically optimized by the ASpect CS software (Analytik Jena AG, Jena, TH, Germany), and the optimized parameters are described in Table I. Considering the fast-sequential capability of the HR-CS FAAS technique, sample throughput was estimated at 48 samples h^{-1}.

Table I. Optimized instrumental operating conditions for the determination of Cu, Fe, K, Mn, Na and Zn by HR-CS FAAS

Analyte	Wavelength (nm)	Acetylene gas flow rate (L h^{-1})	Air flow rate (L h^{-1})	Air/acetylene ratio	Burner height (mm)
Cu	324.754	50	486	9.7	6.0
Fe	248.327	60	486	8.1	6.0
K	404.414	80	486	6.1	8.0
Mn	279.482	80	486	6.1	6.0
Na	330.237	90	486	5.5	6.0
Zn	213.857	50	486	9.7	6.0

Reagents, materials, analytical solutions and samples

All sample digestions and analytical solutions were prepared using ultrapure water (resistivity 18.2 MΩ cm) produced from a Master System MS2000 (Gehaka, São Paulo, Brazil), nitric acid 65% w w^{-1} (Merck, Darmstadt, Germany), and hydrogen peroxide 30% w w^{-1} (Merck, Darmstadt, Germany). For optimizations, 3.5 and 7.0 mol L^{-1} HNO_3 solutions were previously prepared by simple dilution using ultrapure water. High-purity acetylene (99.7%, Air Liquid, São Paulo, Brazil) and compressed air were used as fuel and oxidant gases, respectively. All quartz digestion tubes, glassware, and polypropylene flasks were previously decontaminated by overnight immersion in a 10% v v^{-1} HNO_3 solution, followed by deionized water rinsing.

Multielement standard solutions were prepared by appropriate dilution of respective stock solutions containing 1,000 mg L^{-1} Cu, Fe, K, Mn, Na, or Zn (SpecSol®, QUIMLAB, São Paulo, Brazil). Analytical working solutions in the 0.03 – 0.2 mg L^{-1} Cu, 0.25 – 3.0 mg L^{-1} Fe, 5 – 200 mg L^{-1} K, 0.1 – 0.5 mg L^{-1} Mn, 10 – 50 mg L^{-1} Na and 0.3 – 1.5 mg L^{-1} Zn were daily prepared in a HNO_3 5% v v^{-1} medium. The certified reference materials NIST SRM 1577b Bovine Liver and NIST SRM 2976 Mussel Tissue Freeze-Dried from National Institute of Standards and Technology (Gaithersburg, MD, USA), and MR-E1002A Fish Food from *Empresa Brasileira de Pesquisa Agropecuária* (Embrapa, Brazil) were used to check the accuracy of the procedures. Dry dog foods for puppies, adults and senior animals from 3 different manufacturers were purchased in a local market of Araraquara, SP, Brazil. The samples were ground using a mortar and pestle, and dried at 75 ºC in a TE-394/2 forced air oven (Tecnal, Piracicaba, SP, Brazil) to constant weight for the determination of moisture. Then, dried samples were placed in polypropylene containers and stored in desiccators.

Digestion procedures

Screening study of variables using a factorial design

The screening study of digestion conditions for the CHDS procedure was performed by means of a fractional factorial design with 5 variables (V1 = concentration of HNO_3, V2 = volume of H_2O_2, V3 = holding time, V4 = temperature, and V5 = pre-digestion time) at 2 levels (-1 = low and +1 = high). The real values for the low and high levels were 3.5 and 14.0 mol L^{-1} HNO_3 (V1); 0.5 and 1.0 mL of H_2O_2 (V2); 12 and 24

min (V3); 195 and 225 ºC (V4); and 15 and 30 min (V5), respectively. The results of these experiments were processed using the Statistica (TIBCO Software Inc. Data Science Workbench, version 14, 2020). From this design (2^5), the number of possible combinations would be 32, which was changed to (2^{5-2}) in order to reduce the number of experiments to a total of 8, corresponding to 1/4 fraction of the complete design.[16,17]

In each experiment, masses of 100 mg of dry dog food sample were accurately weighed and 2.0 mL of HNO_3 solution (V1) was added. The vessels were closed and kept at room temperature for the selected pre-digestion time (V5). After that, the selected volume of H_2O_2 (V2) was added and the vessels were sealed. The heating program was initiated using a 20 min ramp up to the chosen temperature (V4) and, after reaching a plateau, this temperature was kept for the selected holding time (V3). After cooling down for 30 min, the resulting solutions were transferred to polypropylene tubes and the final volume was made up to 25 mL with ultrapure water. RCC determinations were performed in duplicates with pH adjusted to a range of 5.5 to 6.5 with NaOH and a 100-fold dilution was made. A summary of the factorial design parameters is depicted in Table II.

Table II. Factorial design (2^{5-2}) for the CHDS digestion optimization

Experiment	Level (real value)				
	V1 HNO_3 (mol L^{-1})	**V2 H_2O_2 (mL)**	**V3 Holding time (min)**	**V4 Temperature (ºC)**	**V5 Pre-Digestion time (min)**
1	-1 (3.5)	-1 (0.5)	-1 (12)	1 (225)	1 (30)
2	-1 (3.5)	-1 (0.5)	1 (24)	1 (225)	-1 (15)
3	-1 (3.5)	1 (1.0)	-1 (12)	-1 (195)	1 (30)
4	-1 (3.5)	1 (1.0)	1 (24)	-1 (195)	-1 (15)
5	1 (14.0)	-1 (0.5)	-1 (12)	-1 (195)	-1 (15)
6	1 (14.0)	-1 (0.5)	1 (24)	-1 (195)	1 (30)
7	1 (14.0)	1 (1.0)	-1 (12)	1 (225)	-1 (15)
8	1 (14.0)	1 (1.0)	1 (24)	1 (225)	1 (30)

Optimizations of HNO_3 concentration and sample mass

After the settlement of the optimal CHDS procedure and heating program parameters, the effect of the HNO_3 concentration was further evaluated using the fish food CRM. In this experiment, 100 mg of sample was accurately weighed and 2.0 mL of HNO_3 solution (7.0 or 14.0 mol L^{-1}) along with 1.0 mL of H_2O_2 were added to the digestion vessels. The vessels were sealed and taken to the CHDS system for digestion using the following heating program: i) a 20-min ramp from room temperature to 195 ºC, ii) a 12-min plateau at 195 ºC and iii) a 30-min cooling down to 40 ºC. The resulting solutions were transferred to polypropylene tubes and the final volume was made up to 25 mL with ultrapure water.

The effect of sample mass amount in the CHDS digestions was also evaluated using the fish food CRM. In this case, sample masses of 50, 100, or 200 mg were weighed and the mixture of 2.0 mL of concentrated HNO_3 and 1.0 mL H_2O_2 was added to the quartz tubes. The samples were digested using the same heating program described in the previous experiment. The resulting solutions were transferred to polypropylene tubes and diluted to 25 mL with ultrapure water. The digested samples in both experiments (n=3) were analyzed by HR-CS FAAS for the determination of macro and micronutrients in the fish food CRM.

Digestion of CRMs and dog food samples

After the CHDS optimizations, 10 samples of dry dog food and 3 certified reference materials were digested (n=3). Sample masses of 200 mg were directly weighed in the CHDS quartz tubes, followed by the addition of 2.0 mL of concentrated HNO_3 and 1.0 mL of H_2O_2. The samples were processed through the same heating program described in section 2.3.2, which consisted of the following 3 steps: a 20-min ramp from room temperature to 195 ºC (step1), a 12-min plateau at 195 ºC (step 2) and a 30-min cooling down to 40 ºC (step 3). The digested samples were then diluted to 25 mL with ultrapure water.

For comparative purposes, all dog food samples were digested using a closed-vessel microwave-assisted digestion system (n=3). Sample masses of 200 mg were transferred to the microwave vessels and a mixture of 2.0 mL HNO_3 (concentrated), 3.0 mL water, and 1.0 mL H_2O_2 were added to react. The vessels were placed to digest under the following heating program: i) 100-500 W ramp lasting for 5 min, ii) 800 W plateau for 15 min, and iii) 0 W cooling down for 15 min. The final volume was made up to 25 mL with ultrapure water.

RESULTS AND DISCUSSION

CHDS optimizations

A fractional factorial design (2^{5-2}) with 5 variables and 2 levels was used for the optimization of several CHDS parameters as HNO_3 concentration, H_2O_2 volume, time of plateau, digestion temperature and pre-digestion time, and using the measured residual carbon content (RCC) as the target response. From these values, the organic matter decomposition efficiencies (%DE) were calculated using the following relationship: %DE = (TCC – RCC)/(TCC) * 100, where TCC is the total carbon content. In this work, the RCC contents were determined by the carbon analyzer and TCC values were obtained by considering the whole sample mass used in digestion as composed only of carbon, thus the calculated %DE may be considered as an estimated value. Results for RCC and %DE are depicted in Figure 1.

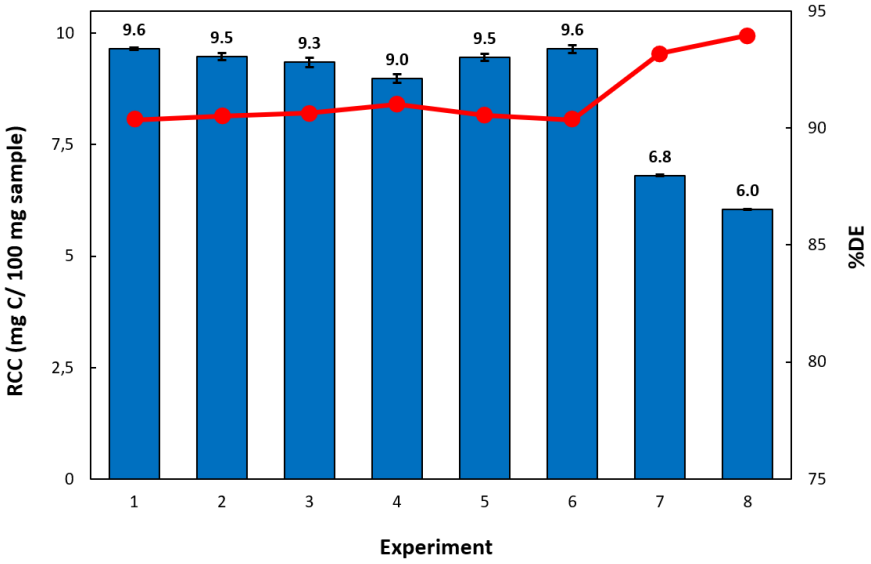

Figure 1. Determination of residual carbon content (RCC, bar chart) and estimated decomposition efficiency (%DE, dot chart) for the designed experiments used to optimize the CHDS digestion parameters.

For all experiments, the digested samples presented a limpid aspect and the residual carbon contents were generally lower than 9.6%. The lowest values for RCC were found in experiments 7 and 8, with carbon contents in the 6.0- 6.8 mg C per 100 mg sample range and estimated %DE higher than 93%. In common, these experiments presented high levels for the variables V1 (14 mol L^{-1} HNO_3), V2 (1.0 mL of H_2O_2), and

V4 (the temperature at 225 ºC). The main differences between experiments 7 and 8 are due to the times of pre-digestion (V5) and plateau (V3), where experiment 8 presented the higher levels for these variables (30 and 24 min, respectively) and experiment 7 the lower levels (15 and 12 min, respectively). These differences may explain the slightly lower levels of RCC for condition 8, as further matrix decomposition was expected with increasing times. A closer look at the significance of the studied variables on the RCC results using a Pareto chart[17] is shown in Figure 2.

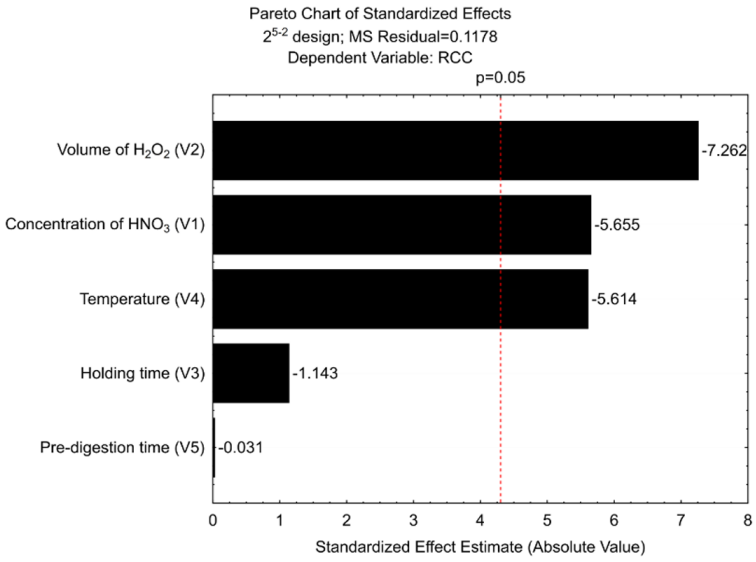

Figure 2. Pareto chart for the influence of variables V1-V5 in the digestion efficiency.

Figure 2 presents a standardized effect estimate for each of the variables in function of the RCC results (dependent variable). The p-value equal 0.05 with a 95% confidence interval corresponds to an effect standardized of 4.3027 (as absolute value). The minus signal of the effects indicates that variables evaluated exert an antagonistic effect under the measured RCC.

The significant variables for CHDS digestions were the volume of H_2O_2, concentration of HNO_3 and the temperature, as the times of pre-digestion and holding during the temperature plateau were not critical. These findings corroborate with the results observed for the pairs of experiments 1-2, 3-4, 5-6, and 7-8, in which the RCC contents are very close and the only difference between themselves was the levels of the time variables (V3 and V5). In this sense, the subsequent experiments were performed without a pre-digestion time and using the lower level of plateau time (12 min). Despite the significance of the digestion temperature and relatively lower values of RCC obtained for 225 ºC, the cooling down step can be performed in shorter times for 195 ºC and lower pressures were achieved, which can improve the safety of the digestion procedure and increase the sample throughput. Thus, the following CHDS experiments were conducted at 195 ºC using 1.0 mL of H_2O_2.

The influence of the HNO_3 concentration on the determinations of Cu, Fe, K, Na, Mn, and Zn by HR-CS FAAS was further investigated using a fish food CRM (MR-E1002A). Residual acidity is a critical parameter for sample introduction in spectrometric techniques, thus the digestions involved 100 mg of the material and were done using 2 concentrations of HNO_3, 7 and 14 mol L^{-1}. Although it was not evaluated in the experimental design, the concentration of 7 mol L^{-1} corresponds to half of the acidity found under the previously optimized conditions. Other optimal parameters obtained using the factorial design (no pre-digestion step, 1.0 mL of H_2O_2, 195 ºC maximum temperature, and a 12-min plateau) were also employed in this experiment and the results are shown in Table III.

Table III. Results (mean ± standard deviation) for the determination of Cu, Fe, K, Na, Mn and Zn in fish food CRM by HR-CS FAAS after CHDS digestions of 100 mg of sample (n = 3) using 7 and 14 mol L^{-1} HNO_3

Analytes	Certified values (mg kg^{-1})	Concentration of HNO_3			
		7 mol L^{-1} (mg kg^{-1})	%Agreement	14 mol L^{-1} (mg kg^{-1})	%Agreement
Cu	10.5 ± 0.9	6.8 ± 0.9	65	8.4 ± 0.5	80
Fe	231.9 ± 20.2	185 ± 12	80	209 ± 5	89
K	5860.0 ± 310.0	4282 ± 130	73	4483 ± 57	77
Mn	19.5 ± 2.1	14 ± 3	72	18 ± 3	93
Na	2160.0 ± 190.0	1876 ± 158	87	1961 ± 7	91
Zn	129.6 ± 6.8	114 ± 5	88	119 ± 2	92

In general, accuracies were better when 14 mol L^{-1} was used for the CRM digestion, with quantitative apparent recoveries in the 77-93% range. On the other hand, for experiments using 7 mol L^{-1} HNO_3, apparent recoveries lower than 75% were found for Cu, K and Mn, which could be due to the greater presence of matrix as the RCC was higher than that found for 14 mol L^{-1} HNO_3. The influence of the sample masses on the CHDS digestions of the fish food CRM (MR-E1002A) and HR-CS FAAS determinations was also investigated (Table IV).

Table IV. Results (mean ± standard deviation) for the determination of Cu, Fe, K, Na, Mn and Zn in fish food CRM by HR-CS FAAS after CHDS digestions (n= 3) of sample masses of 50, 100 and 200 mg using 14 mol L^{-1} HNO_3

Analytes	Certified values (mg kg^{-1})	Sample masses					
		50 mg	Agreement (%)	100 mg	Agreement (%)	200 mg	Agreement (%)
Cu	10.5 ± 0.9	7.6 ± 1.5	72	8.4 ± 0.5	80	8.5 ± 0.1	81
Fe	231.9 ± 20.2	208 ± 38	90	209 ± 5	90	209 ± 2	90
K	5860 ± 310	3563 ± 143	61	4483 ± 57	77	5488 ± 11	94
Mn	19.5 ± 2.1	13.6 ± 0.9	70	18 ± 3	92	20 ± 7	103
Na	2160.0 ± 190.0	1833 ± 67	85	1961 ± 7	91	2188 ± 81	101
Zn	129.6 ± 6.8	113.5 ± 0.8	88	119 ± 2	92	122.1 ± 0.7	94

In this case, the digestions of 50, 100, and 200 mg of the material were evaluated and the concentration of HNO_3 was 14 mol L^{-1}. The same optimized parameters involving 1.0 mL of H_2O_2, temperatures up to 195 ºC and a 12 min hold during the heating program were employed. In general, average recoveries of 94, 87, and 78% were obtained for the digestion of 50, 100, and 200 mg of CRM, respectively. For 50 mg, the accuracies were systematically lower, and the apparent recoveries were situated in the 61 – 90% range. This may be explained by the use of mass amounts lower than recommended by the CRM manufacturer, which can impair the homogeneity and representativeness of samples. The best results were found using 200 mg of sample, thus this mass was employed through the next experiments.

Analytical performance and sample analysis

The trueness and precision for the determination of Cu, Fe, K, Na, Mn, and Zn by HR-CS FAAS using the optimal procedure were checked by analyzing the CRMs of fish food (MR-E1002A), NIST SRM 1577b Bovine Liver and NIST SRM 2976 Mussel Tissue Freeze-Dried (Table V). These materials were selected because of the similarity between the matrices, as dry dog foods are mainly composed by animal protein and fat sources.

Table V. Results (mean ± standard deviation) for the determination of Cu, Fe, K, Na, Mn and Zn in CRMs by HR-CS FAAS after CHDS digestions (n = 3)

Samples		Analytes					
		Cu	Fe	K	Mn	Na	Zn
MR-E1002A Fish Food	Certified value (mg kg^{-1})	10.5 ± 0.9	231.9 ± 20.2	5860 ± 310	19.5 ± 2.1	2160 ± 190	129.6 ± 6.8
	Determined (mg kg^{-1})	8.5 ± 0.1	209 ± 2	5488 ± 11	20 ± 7	2188 ± 81	122.1 ± 0.7
	Agreement (%)	81	90	94	103	101	94
NIST SRM 1577b Bovine Liver	Certified value (mg kg^{-1})	160 ± 8	184 ± 15	9940 ± 20	10.5 ± 1.7	2420 ± 60.0	127 ± 16
	Determined (mg kg^{-1})	124 ± 1	149.7 ± 0.2	7811 ± 95	8.8 ± 0.1	2638 ± 71	124 ± 2
	Agreement (%)	78	81	79	84	109	98
NIST SRM 2976 Mussel Tissue Freeze-Dried	Certified value (mg kg^{-1})	4.0 ± 0.3	171.0 ± 4.9	9700 ± 500	33 ± 2	35000 ± 1000	137 ± 13
	Determined (mg kg^{-1})	3.8 ± 0.1	142.9 ± 0.3	7792 ± 67	29.3 ± 0.1	33131 ± 227	135 ± 4
	Agreement (%)	95	84	80	89	95	99

Considering all the CRMs and analytes, quantitative results were typically obtained, for Fish Food (MR - E1002A) recoveries between 81 and 103% were obtained with RSDs in the range of 0.2 at 1.2% (excluding the 35% of uncertainty observed for Mn), Bovine Liver (NIST SRM 1577b) that showed recoveries of 78 to 109% with RSDs between 0.1 and 2.7% and, Mussel Tissue Freeze-Dried (NIST SRM 2976) with recoveries in the range of 80 at 99% and RSDs from 0.3 to 3.0%. Precisions as relative standard deviations were generally better than 3.0%, and the overall average precision was around 1.6%, except for the measures of Mn in Fish Food that shown 35% of uncertainty in this determination, however in the CRM a value greater than 10% was also shown for this analyte. Considering these results, the combination of CHDS for sample digestions and HR-CS FAAS for determination provided adequate results for the analysis of animal protein matrices.

The found values for the analytes in the CRMs were checked by unpaired *t*-test against the certified values, statistical comparison shown a significant difference between these data, which can be explained considering that the standard deviation of the digested measurements was for several samples much lower than the uncertainty presented in the certificate. Only approximately 30% of values tested by unpaired *t*-test were statistically concordant with the certified, however, the found recoveries shown a great accuracy for 72% these data considering an interval from 84 to 110% of recovery.

Figure 3 summarizes the results data presented in the Table V, are plotted the found values after CHDS digestion and HR-CS FAAS determination versus the certified values for all CRMs (and analytes), in this figure it can be observed that the obtained measurements were really concordant with the certified values in these sample matrices.

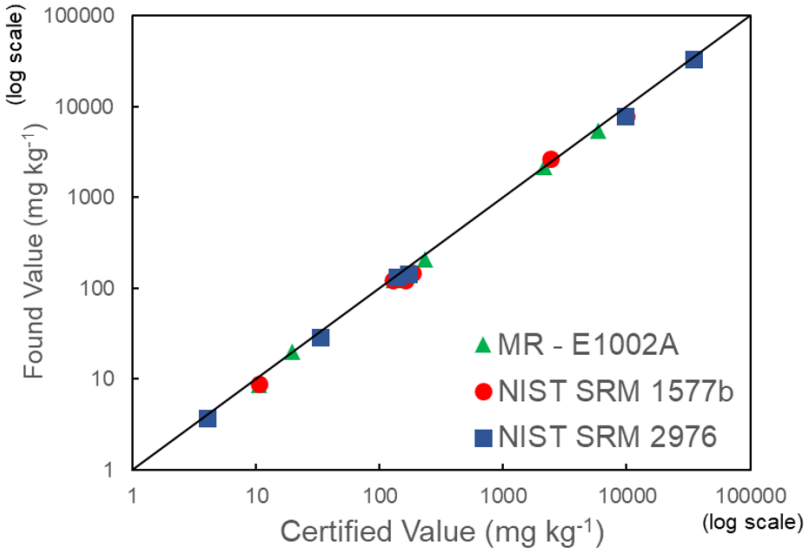

Figure 3. Comparison between found values obtained by FAAS after CHDS digestion of certified materials and the certified reference values.

The developed procedure was then employed for the analysis of 10 real samples of dog food and a comparison between the well-established microwave-assisted digestion (MW-AD) and the proposed conductively heated digestion system (CHDS), both using closed vessels, was performed (Table VI). For macronutrients (Na and K), the determined concentrations were in the 3221 – 6057 mg kg^{-1} range and for micronutrients (Cu, Fe, Mn, and Zn) the contents varied from 8 to 255 mg kg^{-1}. In general, the MW-AD and CHDS methods were comparable, and no statistical differences were found at a 95% confidence level (paired *t*-test) in all cases. Moisture is an important quality parameter which can impact shelf-life of dog foods. Determined values for moisture were in the 3.7 – 6.1%, which are lower than 12% and may be considered adequate.[21]

Table VI. Results for moisture and digestions by CHDS and MW-AD (n= 3) for the determination (mean ± standard deviation) of Cu, Fe, K, Na, Mn, and Zn in dry dog food samples by HR-CS FAAS and *t*-test values (*t*-critical = 4.303)

Sample	Moisture (%)	Digestion procedure	Analytes (mg kg^{-1})					
			Cu	Fe	K	Mn	Na	Zn
1	5.4	MW-AD	23.4 ± 0.1	203 ± 2	5095 ± 67	61 ± 2	5693 ± 122	253 ± 1
		CHDS	23.3 ± 0.1	235 ± 2	4828 ± 230	59.8 ± 0.4	5691 ± 282	255 ± 8
		t-value	0.66	2.85	1.58	0.47	0.01	0.39
2	4.7	MW-AD	10.1 ± 0.1	174 ± 13	5726 ± 6	38.4 ± 0.6	4094 ± 162	134 ± 2
		CHDS	10.4 ± 0.3	181 ± 8	5724 ± 48	38.4 ± 0.9	4240 ± 18	128 ± 7
		t-value	1.75	0.71	3.63	0.05	1.21	3.16
3	3.7	MW-AD	29 ± 1	201 ± 4	5084 ± 179	30.5 ± 0.2	3282 ± 99	227 ± 2
		CHDS	30 ± 1	223 ± 3	5038 ± 70	30.6 ± 0.3	3221 ± 94	229 ± 1
		t-value	2.94	3.14	0.33	0.26	0.68	1.48

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Table VI. Results for moisture and digestions by CHDS and MW-AD (n= 3) for the determination (mean ± standard deviation) of Cu, Fe, K, Na, Mn, and Zn in dry dog food samples by HR-CS FAAS and *t*-test values (*t*-critical = 4.303) (continuation)

Sample	Moisture (%)	Digestion procedure	Analytes (mg kg^{-1})					
			Cu	Fe	K	Mn	Na	Zn
4	6.1	MW-AD	9.4 ± 0.6	176 ± 8	5951 ± 125	42.7 ± 0.6	4264 ± 62	140 ± 4
		CHDS	9 ± 5	201 ± 1	5834 ± 162	42.7 ± 0.3	4141 ± 183	137 ± 3
		t-value	0.06	4.25	2.66	2.39	1.11	0.83
5	5.3	MW-AD	12.6 ± 0.2	209 ± 5	5132 ± 61	41.5 ± 0.4	4988 ± 395	122 ± 3
		CHDS	12.6 ± 0.2	194 ± 6	4958 ± 501	41.2 ± 0.3	5150 ± 107	121 ± 4
		t-value	0.04	0.41	0.65	1.21	0.69	0.06
6	6.3	MW-AD	11.8 ± 0.7	198 ± 4	4728 ± 159	40.6 ± 0.3	4795 ± 132	128 ± 3
		CHDS	12.5 ± 0.3	206 ± 10	4702 ± 56	41 ± 1	4734 ± 45	123 ± 6
		t-value	1.21	2.71	0.26	1.09	0.76	2.52
7	6.0	MW-AD	9.6 ± 0.2	153.3 ± 0.6	5268 ± 157	55.7 ± 0.3	4252 ± 65	169 ± 3
		CHDS	9.3 ± 0.6	163.5 ± 0.2	5281 ± 106	56 ± 1	4175 ± 245	175 ± 8
		t-value	0.98	2.41	2.18	0.27	0.53	1.24
8	5.7	MW-AD	9.4 ± 0.7	149 ± 1	6057 ± 107	46.7 ± 0.5	3811 ± 39	119 ± 3
		CHDS	8 ± 1	144 ± 6	5876 ± 269	48 ± 2	3919 ± 157	118 ± 4
		t-value	1.45	1.86	1.11	1.29	1.16	0.18
9	5.7	MW-AD	11 ± 1	141 ± 6	4753 ± 84	47.2 ± 0.5	4282 ± 31	135 ± 5
		CHDS	10.1 ± 0.6	156 ± 16	4660 ± 106	47.9 ± 0.1	4387 ± 113	138 ± 2
		t-value	3.94	1.29	1.03	1.81	1.55	2.96
10	5.5	MW-AD	11.9 ± 0.5	152 ± 14	5416 ± 80	38.0 ± 0.7	3383 ± 85	140 ± 4
		CHDS	11.6 ± 0.5	157 ± 10	5478 ± 35	40 ± 2	3489 ± 265	140 ± 2
		t-value	0.73	0.47	1.00	1.44	0.66	0.39

The elemental concentration ranges obtained in this work were compared with data from the literature (Table VII). In general, the concentrations of macro and micronutrients in dry dog foods are in the same range as those reported in the literature described in Table VII. Limits of detection (LOD) were calculated according to the IUPAC recommendations, and the measurements were performed using the blank solutions obtained from the MW-AD and CHDS procedure. LODs for MW-AD digestions were 3.4 (Cu), 11 (Fe), 60 (K), 0.5 (Mn), 128 (Na), and 3.5 (Zn) mg kg^{-1}, while for CHDS the values were 3.1 (Cu), 10 (Fe), 56 (K), 0.7 (Mn), 96 (Na), and 3.6 (Zn) mg kg^{-1}. In both cases, the LODs were comparable and low enough to allow the determination of macro and micronutrients in dog food. Both foods intended for dogs and cats are mainly based on animal sources of protein and fat, and the levels of these macromolecules are also very similar. Thus, it is possible to infer that similar performance would be attained for CHDS digestions

of these samples. In addition, pet foods are made primarily from animal protein sources, and the CHDS system has already proven itself as a good alternative for the digestion of this raw materials.[13]

Table VII. Determined concentration ranges for macro and micronutrients for this work and comparison with literature values

Analytes	Determined concentration range (mg kg^{-1})					
	Present work	**Sgorlon et al. 2022[18]**	**Costa et al. 2013[19]**	**Kelly et al. 2013[20]**	**Elias et al. 2012[21]**	**Duran et al. 2010[22]**
Cu	8 – 30	7.0 – 30	15.5 – 34.1	7.7 – 18.0	-	3.3 – 16.6
Fe	141 – 223	50 – 450	147 - 606	56 - 220	188 – 646	23.9 – 71.1
K	4660 – 6057	4000 - 8000	1000 - 1300	-	5290 – 10500	-
Mn	30.5 – 61	20 – 90	6.5 – 149	16 - 70	-	3.3 – 24.4
Na	3221 – 5693	2500 - 7000	-	-	3180 – 7050	
Zn	118 – 255	90 - 550	106 – 419	79 – 330	44 - 633	-

*Estimated from data plots

Sample discrimination

Concerning the dry dog food, samples 3 and 1 were from manufacturers A and B, respectively, and samples 2, 4, 5, 6, 7, 8, 9, and 10 were from manufacturer C. As for the animal life stage, samples 1, 3, 5, 6, and 7 were destined for puppies, samples 2, 4, 8, and 9 for adults and sample 10 for senior dogs. As the formulation of minerals may vary according to different manufacturers and the needs for nutrients at different dog's life stages, the determined contents of moisture, Cu, Fe, K, Na, Mn, and Zn using CHDS and HR-CS FAAS were used for the proposition of hierarchical cluster analysis (HCA), aiming for the discrimination between the samples (Figure 4). Samples discrimination may be considered an important way to check for food fraud or mislabeling, for example.[24] From the dendrogram, it is possible to check that samples may be accurately separated into three distinct groups according to their manufacturer. From the 8 samples from manufacturer C (red lines), samples 2, 4, 8, and 10 are related to adult or senior dogs and were clustered at a distance equal to 2. Moreover, samples 5, 6 and 7 are related to the puppy life stage and were also grouped at the same distance. On the other hand, sample 9 which corresponds to adult dog food was incorrectly grouped as puppy food. From the results in Table VI, it is possible to confirm that the contents found for K, Mn, Na, and Zn in sample 9 were closer to the averages of the puppy food group than the adult food group, thus explaining the incorrect clustering for this sample.

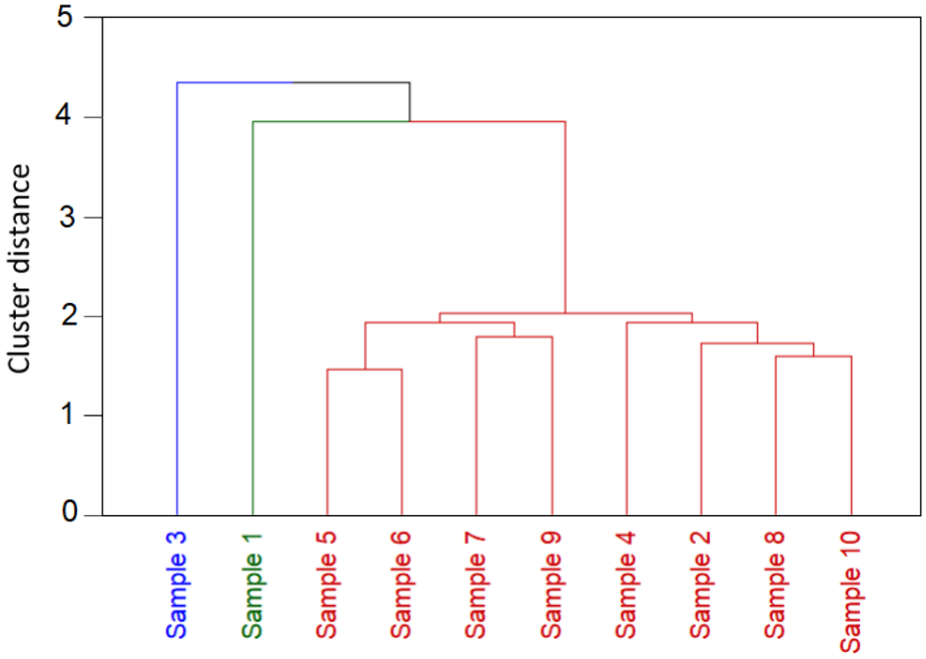

Figure 4. Hierarchical clustering dendrogram using mineral composition and moisture data from dog food samples from manufacturer A (blue), manufacturer B (green) and manufacturer C (red).

CONCLUSIONS

The application of a factorial design for CHDS digestion allowed the execution of only 8 experiments to obtain the optimal conditions as 14 mol L^{-1} HNO_3, 1.0 mL of H_2O_2, 195 ºC of maximum temperature, 12 min of holding time, and no need for a pre-digestion step for the decomposition of dog food samples. In spite of the greater efficiency of decomposition at 225 ºC, safety conditions and sample throughput were incremented when 195 ºC was used. For all tested conditions, the volume of H_2O_2, concentration of HNO_3 and temperature parameters were the most significant and the estimated decomposition rates were better than 90%. Furthermore, the analysis of CRMs from animal food evidenced that the use of 14 mol L^{-1} HNO_3 for the digestion of 200 mg of sample provided the best accuracies. Under the optimal conditions, accurate determinations of Cu, Fe, K, Na, Mn, and Zn by HR-CS FAAS were achieved for three CRMs from animal protein sources. The developed CHDS procedure and a comparative MW-AD were applied to 10 real samples from dry dog food and the results showed that both were statistically equivalent at a 95% confidence level. Limits of detection calculated using the blank solutions from CHDS and MW-AD were comparable and adequate for the determination of macro and micronutrients in dog food. Although the performances proved to be equivalent in terms of efficiency, preparation time and generation of residues, CHDS may be considered simpler and less expensive than MW-AD. The hierarchical cluster analysis using the moisture and mineral composition results demonstrated similarities for samples from different manufacturers and, in most cases, for samples destined to different animal life stages for the same manufacturer. The HCA has proven to be a valuable tool for samples discrimination, with potential applications in checking for tampering and fraud in dry dog foods. The procedure combining CHDS digestion and HR-CS FAAS determination provided sensitive, accurate and precise analytical conditions, being a good alternative for dog food quality control

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

The authors thank "Fundação de Amparo à Pesquisa do Estado de São Paulo" (FAPESP) [grants 2019/07537-6, 2020/00907-0 and 2021/01912-0] and "Conselho Nacional de Desenvolvimento Tecnológico" (CNPq) [grant 303607/2021-1]

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