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Microwave-Assisted Acid Decomposition of Animal- and Plant-Derived Samples for Element Analysis

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A single microwave-assisted acid-decomposition procedure is proposed for sample preparation of plant- and bovine-derived materials prior to multielement determination by inductively coupled plasma−optical emission spectrometry (ICP−OES). The procedure involved sample grinding in a cryogenic mill to reduce particle sizes and increase the surface area for acid attack, followed by decomposition under high pressure and temperature. A single heating program was employed and efficiently destroyed most of the organic matrix by using small volumes of nitric acid and hydrogen peroxide. Accuracy of the elemental analysis was indicated by the good agreement between certified and found values of elements in plant and animal samples at 95% confidence level. The sample preparation procedure was fast and reproducible, and proved to be efficient for elemental analysis in agricultural and food-related samples by a sensitive multielement technique such as ICP−OES.

KEYWORDS: Animal and plant samples; microwave-assisted decomposition; cryogenic grinding; main, minor and trace elements; ICP−OES

INTRODUCTION

Minerals are required by beef cattle and can directly affect productivity. Macrominerals required include Ca, Mg, P, K, Na, Cl, and S, and the microminerals are Cr, Co, Cu, I, Fe, Mn, Mo, Ni, Se, and Zn. Others, including As, B, Pb, Si, and V, have been shown to be essential for one or more animal species (*1*), but there is no evidence that these minerals are of practical importance in beef cattle. Usually, many of the essential minerals are found in appropriate concentrations in feedstuffs. However, others are many times insufficient in diets fed to cattle. In this case, supplementation is necessary to improve animal performance, health conditions, and productivity. The determination of mineral contents in beef cattle diet and in selected parts of these animals allows the assessment of the nutritional requirements of these animals and helps to efficiently use the resources in the production system. In beef cattle production, this evaluation is important because of the high cost of animal nutrition (sometimes reaching up to 70% of total production cost). The determination of some other elements, which are usually not required and can cause toxicity to beef cattle and meat consumers, is also important to assess beef quality. Aluminum and Cd are among those elements considered toxic if present in concentrations above that tolerable for cattle (*2*). The maximum tolerable concentration for a mineral has been defined as "that dietary level that, when fed to an animal for a limited period, will not impair animal performance and should not produce unsafe residues in human food derived from the animal" (*3*).

A sample pretreatment step is generally necessary in the majority of the analytical methods. In elemental analysis, most of the analytical techniques require the solid sample to be transformed into a homogeneous liquid phase. Microwave-based techniques have been efficiently used in the decomposition of a variety of samples for elemental analysis. Microwave energy as the heat source in acid decomposition was first proposed by Abu-Samra et al. (*4*) who reported its application to wet digestion of biological samples. This method, which is now widely used in elemental analysis, involves the total or partial decomposition of the sample, destroying most of the organic matter (*5*). In comparison with conventional methods of sample digestion, these systems reduce contamination and increase the efficiency of the decomposition process (*6*). In procedures using microwave energy, the decomposition temperature required is reached in 5 to 10 min, unlike in conductive heating in which longer heating times are necessary. In closed vessel systems under high temperature and pressure, total metal recovery is achieved for volatile elements and there is also no risk of

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contamination from the environment or other samples. Low blank values are usually obtained due to the small amounts of the reagents used for digestion.

Another important parameter in the efficiency of sample decomposition and in the final analysis is the material particle size. Grinding the samples prior to digestion allows faster decomposition and better homogenization, leading to more precise results. The choice of an appropriate grinder for a specific sample depends on the goals of the analysis, and one must consider the type and possible contamination according to the material investigated. The commercial cryogenic grinder is an efficient alternative for achieving small sample particles (*7*). A recent work (*8*) on sample grinding has reported the efficiency of this apparatus to reduce particle sizes of food samples up to 100 μm.

Inductively coupled plasma−optical emission spectrometry (ICP−OES) has been extensively used for multielemental analysis due to its low detection limits, large linear dynamic range, and high precision (*9*−*11*). Compared to other sources of atomic excitation, ICP−OES shows the advantages of a multielemental determination with high analytical frequency and the versatility to be interfaced with other analytical techniques. The selectivity of ICP−OES in analytical spectrometry makes it suitable for elemental analysis in a variety of different matrixes for simultaneous or sequential metal determination in a wide concentration range. The high signal-to-background ratio characteristic of the ICP−OES generates the technique's low detection limits (*11*).

This work proposes a microwave-assisted acid decomposition procedure for plant- and animal-derived samples using a single heating program prior to element determination by ICP−OES. Samples were subjected to cryogenic grinding prior to digestion, and various parameters responsible for the method efficiency were investigated in routine analysis.

MATERIALS AND METHODS

Apparatus. A model 6750 Spex freezer/mill (Spex CertiPrep, Metuchen, NJ) with a self-contained liquid nitrogen bath was used with model 6751 grinding vials. Sample decomposition was assisted in a closed vessel microwave oven, ETHOS 1600 (Milestone, Italy). A Labconco freeze-drier (Kansas City, MO) was used to lyophilize all bovine samples. A simultaneous atomic emission spectrometer ICP−OES VISTA RL (Varian, Australia) with radial view configuration was used for elemental determination. The plasma was operated with a concentric nebulizer coupled to a cyclone type nebulization chamber (Glass Expansion, Australia). An element analyzer CHNS-O EA 1108 (Fisons Instruments, Italy) was used for the determination of total carbon.

Reagents and Samples. Distilled, deionized water of 18 MΩ-cm resistivity, obtained from a Milli-Q system (Millipore, Bedford, MA) was used to prepare all solutions. Reagent grade HNO_3 (Carlo Erba, Italy) and 30% v/v H_2O_2 (Mallinckrodt, Mexico) were used for acid digestion of the samples. Spex plasma standard (1000 mg L^{-1}) was used to prepare Al, Ba, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, S, Se, V, and Zn reference solutions which ranged from 2.5 to 100 mg L^{-1}. Carbon reference solutions ranging from 50 to 200 mg L^{-1} were prepared from a 2000 mg L^{-1} urea stock solution (Reagen, Brazil). Liquid nitrogen was used for sample freezing in the cryogenic grinding step. All glass and plasticware was washed with diluted neutral cleaning solution, soaked overnight in 10% v/v nitric acid, and rinsed 3 times with deionized water before use.

A variety of animal-derived samples was selected as indicators of the nutritional level of crossbred $^1/_2$Nelore + $^1/_2$Angus young bulls, which were fed on coast cross for 12 months. The animals were slaughtered and divided into six parts including hide, viscera, carcass, blood, ribs, and head and feet, according to the methodology proposed by Luchiari and co-workers (*12*). Prior to grinding, these samples were lyophilized in glass flasks connected to the vacuum system of the freeze-drier, which operated at −150 °C for 80 h, and then stored under −10 °C. The forage (coast cross) sample was oven-dried at 65 °C for 48 h followed by cryogenic grinding. Certified bovine liver (National Institute of Standard and Technology, NIST 1577b), apple leaves (NIST 1515), and bovine muscle (NIST 8414) were used for checking accuracy and method validation.

Table 1. Closed-Vessel Microwave Program for Sample Digestion

stage	power (W)	temperature[a] (°C)	time (min)
1	250	120	2
2	0		3
3	550	180	4
4	650	240	5
5	750	240	5
vent			5

[a] Estimated temperature inside the reaction vessel.

Grinding and Drying Procedures. All samples were ground in the impact freezer/mill, which operates at liquid nitrogen temperatures (−195.8 °C) and effectively pulverizes solid materials. During operation, the grinding vial, a polycarbonate cylinder supplied with two stainless steel end plugs, is immersed in liquid nitrogen. An alternating magnetic field shuttles a steel impactor against the ends of the vial, powdering the coarse sample. The sample amount used varied from 1 to 2 g. In a full-day operation the mill consumed around 20 L of liquid nitrogen. Each sample was precooled for 5 min and then ground for 2 min. Three grinding cycles were performed and a cooling step of 1 min was applied between each cycle.

After grinding, the moisture content of each sample was determined. Approximately 2 g of each animal-derived material and the coast cross sample were placed in an oven operating at 105 °C for approximately 8 h. The samples were weighed between hours until constant mass was acheived.

Microwave-Assisted Acid-Decomposition. To convert the samples into solutions for elemental analysis, microwave-assisted acid decomposition was performed at high pressure and temperature. A preliminary digestion was performed at the conditions recommended by the instrument manufacturer, which employed 250 mg of sample and 5 mL of 65% v/v HNO_3 plus 2 mL of 30% v/v H_2O_2. After an optimization study for the decomposition process (results not shown), the reagents mixture was set as 2 mL of concentrated HNO_3 plus 1 mL of 30% v/v H_2O_2. Sample mass was 300 mg of animal-derived materials and 250 mg of forage, which were directly weighed in perfluoralkoxy (PFA) vessels of the microwave oven. After completing the digestion program, the vessels were cooled, and the digests were transferred to 25-mL volumetric flasks and diluted with distilled−deionized water. The microwave operational parameters including temperature, power, and time are listed in **Table 1**. In each decomposition cycle, 10 vessels (1 blank and 9 samples) were used simultaneously.

Element Determination by ICP−OES. Element concentration in the samples was determined by ICP−OES. The glass concentric nebulizer and the cyclone nebulization chamber allowed less dispersion of the sample and decreased the washing step. **Table 2** lists the operational parameters of the spectrometer. Three emission lines (atomic and ionic) for each element were tested before selection. The choice of the analytes' spectral lines was based on both their sensitivity and spectral interference.

RESULTS AND DISCUSSION

Moisture Content. The percentage of moisture in the samples allowed the correction of the sample mass by subtracting the moisture content in each sample from the total weight, which gave the actual mass of dry material. The moisture content in lyophilized hide, head/feet, carcass, ribs, viscera, blood, and coast cross after grinding was 9.5, 8.5, 7.0, 6.1, 4.2, 1.9, and 6.0%, respectively. The variation in moisture content in the samples indicates the importance of determining the mass of

Table 2. Operational Parameters for ICP–OES Measurements

power (kW)	1.3
plasma gas flow (L min^{-1})	15
auxiliary gas flow (L min^{-1})	1.5
nebulizer gas flow (L min^{-1})	0.7
observation height (mm)	8
sample uptake rate (mL min^{-1})	0.8
analytical wavelength (nm)[a]	
Al II	167.019
B I	249.772
Ba II	455.421
C I	193.027
Ca II	317.938
Cd II	226.502
Co II	228.615
Cr II	267.716
Cu I	327.395
Fe II	238.205
K I	766.507
Mg II	280.275
Mn II	257.610
Na I	588.988
Ni II	231.607
P I	177.433
S I	181.972
Se I	196.026
V II	292.406
Zn I	202.549

[a] I and II, atomic and ionic emission line, respectively.

Table 3. Original Carbon Content in the Samples and Residual Carbon after Microwave-Assisted Acid Decomposition

	(%)	
sample	original C[a]	residual C[b]
coast cross	42	3.7 ± 0.3
hide	51	6.6 ± 0.6
carcass	50	9.0 ± 1.5
blood	50	9.8 ± 0.4
ribs	48	11.9 ± 1.7
head/feet	33	6.1 ± 0.4
viscera	64	17.2 ± 0.1

[a] Relative standard deviation around 5%. [b] Mean values ± standard deviation ($n = 3$), expressed as a percentage of the original carbon content in the sample.

dry material in order to improve accuracy and precision of the analysis. Moisture content in the certified materials was around 4% in bovine muscle and liver, and 3% in apple leaves.

Microwave-Assisted Acid-Decomposition Associated with Cryogenic Grinding. Sample decomposition was greatly improved by grinding the materials in the cryogenic mill. The particle size reduction increases sample surface area allowing better acid attack. This grinding procedure produces more uniform particles in the final homogenate, which enhances the method precision. The relative standard deviations (RSD) between replicates of more coarse samples such as carcass, ribs, and head/feet decreased when these samples were cryogenic-ground prior to digestion. For the majority of the elements determined by ICP−OES, the RSD of three replicates was as high as 12% for unground samples. These values decreased significantly reaching up to 1% for cryogenic-ground material. Even though contamination of the sample with Cr, Fe, and Ni from the container's impactor and end plugs had been reported in fish analysis (*7*), no significant differences between certified and found element contents were observed in the analysis of cryogenic-ground SRM bovine liver (NIST 1577b). Because these contaminants (except Fe) are present at levels below the detection limits of the spectrometer, contamination cannot be fully assessed. However, we can suggest from the results of certified and found values of Fe (**Table 4**) that no contamination of this metal took place. Additionally, the contamination degree usually depends on both the sample hardness and the degradation of the stainless steel impactor and end plugs. In the present work, a brand-new cryogenic grinder was employed and the samples were soft materials easily ground.

Depending on the efficiency of the digestion method, a significant amount of carbon residue can remain after decomposition, causing interference such as an increment in optical background, which can lead to interference in the element emission lines. **Table 3** shows the results of total carbon content before digestion and the residual carbon (RC) in each sample after microwave-assisted acid-decomposition. The proposed single heating program allowed efficient decomposition of materials of different composition, eliminating most of the organic matter, indicated by the low carbon content (from 4.0 to 17%) remaining in the digests. The amount of RC in the digestion solutions determined by ICP−OES seemed to be related to the type of carbon present in the sample and apparently did not depend on the amount of original carbon. A fair correlation between remaining carbon after microwave-assisted decomposition and the fat content in these samples has been recently demonstrated (*13*). Decomposition temperatures for some matrix constituents of biological and botanical samples

Table 4. Element Contents (μg g^{-1}, dry basis) in Animal- and Plant-Derived Samples Determined by ICP–OES[a]

sample	Al	B	Ba	Cu	Fe	Mn	Zn
hide	112 ± 9	< 9.5	< 7.7	6.3 ± 0.2	188 ± 2	< 5.0	16.4 ± 1.9
viscera	52.0 ± 0.6	< 9.5	< 7.7	12.2 ± 0.7	383 ± 6	8.7 ± 1.5	47.2 ± 0.6
carcass	< 10	< 9.5	< 7.7	4.1 ± 1.6	65.0 ± 3.0	< 5.0	89.5 ± 1.1
blood	< 10	< 9.5	< 7.7	6.7 ± 0.3	1519 ± 56	< 5.0	18.8 ± 6.5
ribs	< 10	< 9.5	19.2 ± 0.2	13.9 ± 0.2	81.6 ± 2.4	< 5.0	86.4 ± 0.9
head/feet	< 10	< 9.5	54.8 ± 0.4	4.5 ± 2.8	77.4 ± 1.6	< 5.0	51.6 ± 1.3
coast cross	58.9 ± 2.8	6.3 ± 0.9	8.3 ± 0.5	7.3 ± 1.4	98.9 ± 0.6	77.5 ± 3.3	16.0 ± 2.4
bovine muscle[b]							
certified	1.7 ± 1.4	0.6 ± 0.4	0.05[c]	2.84 ± 0.45	71.2 ± 9.2	0.37 ± 0.09	142 ± 14
found	< 10	< 9.5	< 7.7	3.10 ± 0.51	63 ± 3	< 5.0	126 ± 7
bovine liver[b]							
certified	3[c]			160 ± 8	184 ± 15	10.5 ± 1.7	127 ± 16
found	< 10	< 9.5	< 7.7	153 ± 6	171 ± 11	8.8 ± 0.7	114 ± 5
apple leaves[b]							
certified	286 ± 9	27 ± 2	49 ± 2	5.64 ± 0.24	83 ± 5	54 ± 3	12.5 ± 0.3
found	270 ± 6	25 ± 1	51 ± 1	6.60 ± 1.10	71 ± 4	48 ± 2	11.1 ± 0.9

[a] Mean values ± standard deviation ($n = 3$). [b] Standard reference material. [c] Noncertified values.

Table 5. Element Contents (mg g^{-1}, dry basis) in Animal- and Plant-Derived Samples Determined by ICP–OES[a]

sample	Ca	K	Mg	Na	P	S
hide	0.304 ± 0.002	2.50 ± 0.11	0.080 ± 0.003	4.50 ± 0.19	1.40 ± 0.03	5.12 ± 0.15
viscera	1.29 ± 0.02	5.42 ± 0.14	0.278 ± 0.001	1.89 ± 0.07	4.49 ± 0.03	2.96 ± 0.03
carcass	39.5 ± 0.9	8.21 ± 0.12	1.08 ± 0.02	2.38 ± 0.03	22.7 ± 0.6	4.27 ± 0.04
blood	0.490 ± 0.020	3.60 ± 0.01	0.080 ± 0.005	12.6 ± 0.4	1.03 ± 0.01	8.18 ± 0.02
ribs	35.8 ± 0.4	7.18 ± 0.29	0.960 ± 0.008	1.87 ± 0.06	20.8 ± 0.3	3.71 ± 0.08
head/feet	127 ± 1	2.04 ± 0.01	2.41 ± 0.01	4.64 ± 0.04	57.7 ± 2.5	2.17 ± 0.01
coast cross	2.63 ± 0.20	22.4 ± 0.2	1.96 ± 0.05	0.020 ± 0.001	1.65 ± 0.03	2.56 ± 0.20
bovine muscle[b]						
certified	0.145 ± 0.020	15.2 ± 0.4	0.960 ± 0.095	2.10 ± 0.08	8.36 ± 0.45	7.95 ± 0.41
found	0.119 ± 0.007	16.8 ± 0.2	0.920 ± 0.033	1.99 ± 0.11	7.64 ± 0.37	7.26 ± 0.50
bovine liver[b]						
certified	0.116 ± 0.004	9.94 ± 0.02	0.601 ± 0.028	2.42 ± 0.06	11.0 ± 0.3	7.85 ± 0.06
found	0.109 ± 0.005	9.28 ± 0.42	0.588 ± 0.018	2.35 ± 0.11	10.4 ± 0.6	7.16 ± 0.56
apple leaves[b]						
certified	15.3 ± 0.2	16.1 ± 0.2	2.71 ± 0.08	0.024 ± 0.001	1.59 ± 0.11	1.8[c]
found	14.9 ± 0.2	15.3 ± 0.8	2.64 ± 0.06	0.022 ± 0.002	1.50 ± 0.01	1.62 ± 0.04

[a] Mean values ± standard deviation ($n = 3$). [b] Standard reference material. [c] Noncertified values.

have been described elsewhere (*14*), and closed-vessel microwave-assisted digestion has been reported to achieve temperatures above them by using only nitric acid (*15*). These results help in demonstrating the efficiency with which animal and plant samples were dissolved in the present work employing only a single digestion procedure with small amounts of nitric acid and hydrogen peroxide. These parameters were optimized along with the residual carbon content in the digestates to establish the best decomposition temperature for all samples.

Element Determination by ICP−OES. The results from elemental analysis by ICP−OES are presented in **Tables 4** and **5**, which list the contents of minor and major elements, respectively. The detection limit (DL) of all elements was calculated by DL = ks_B/S, where k is equal to 3 (for a 95% confidence level), s_B is the standard deviation of 10 measurements of the blank, and S is the angular coefficient of the analytical curve. The concentrations of trace elements such as Cd, Co, Cr, Ni, Se, and V in the samples were below their DLs (2.7, 4.0, 2.2, 6.0, 3.5, and 4.5 μg g^{-1}, respectively). These results also indicate that reduced amounts of these elements are present in the studied samples. As can be seen in **Table 4**, for most of the bovine samples the contents of Al, B, Ba, and Mn were also below the DLs, which are indicated as <DL. For the remaining elements, C, Ca, Cu, Fe, K, Mg, Na, P, S, and Zn, the DLs were 2500, 11, 6.0, 8.5, 24, 9.6, 65, 80, 35, and 4.0 μg g^{-1}, respectively. These values were not significantly affected by the residual carbon contents in the samples. The relatively high Al concentration in hide may be due to soil contamination as the presence of this metal in these samples is not expected at this level. The poor control during manipulation of the animals prior to analysis may introduce some source of contamination, which is in most of the cases out of the analyst's control. Most of the element contents found are in agreement with those usually expected in beef cattle fed on a diet based on forage (*16*). Certified and found element contents in bovine liver, bovine muscle, and apple leaves, also shown in **Tables 4** and **5**, exhibited a fair agreement between the two values at a 95% confidence level. In view of these results, the procedure used for sample decomposition proved to be appropriate for elemental analysis allowing efficient recovery and low RSD exhibited for the majority of the elements.

Microwave-assisted wet decomposition proved to be a suitable procedure in analytical chemistry for sample preparation of a variety of materials. However, most of the applications in sample digestion for elemental analysis involve the use of different heating programs for each type of sample and usually different reagent amounts. The proposed single heating program using microwave energy allowed efficient acid decomposition of materials of different composition, eliminating most of the organic matter. In addition, the single heating procedure provided colorless and homogeneous digests by employing the same amount and type of reagents. It was fast, reproducible, and proved to be efficient in routine analysis for multielement determination by ICP−OES.

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