Analytical Methods

PAPER

Determination of cadmium and lead in fresh meat by slurry sampling graphite furnace atomic absorption spectrometry

Isabel Cristina Ferreira Damin,[a] Ariane Vanessa Zmozinski,[a] Aline Rocha Borges,[a] Maria Goreti Rodrigues Vale[ab] and Márcia Messias da Silva*[ab]

In this work a method for cadmium and lead determination in fresh meat based on slurry sampling graphite furnace atomic absorption spectrometry is proposed. The fresh meat samples were weighed directly into the autosampler cups of the spectrometer using a microbalance and the slurry was prepared using tetramethylammonium hydroxide (TMAH). The use of the response surface method for optimizing the slurry preparation has shown that it was possible to find the optimum zone in the experimental field of the variables under study. Calibration was performed against aqueous standards and a mixture of 0.05% v/v Pd + 0.03% v/v Mg + 0.05% v/v Triton X-100 was used as a modifier. The results obtained for cadmium and lead in two certified reference materials were statistically not different from the certified values on a 95% confidence level, indicating that calibration against aqueous standards is suitable for this application. A comparison of the results obtained by slurry sampling with the digestion method adopted by the Brazilian Ministry of Agriculture showed no significant differences between the results at the 95% confidence level. The positive results obtained in this work suggest that the application of this procedure in other element determinations is worthy of exploration.

1. Introduction

Brazil, as major producer and supplier of some commodities of great importance for many regions of the world, such as meat, has a duty to ensure that products sold are in accordance with the criteria of safety and quality demanded by consumers. An important tool to closely monitor and assure this compliance is the Brazilian National Residue Control Plan.[1]

Meat and meat products constitute an important part of the human diet and the content of toxic metals such as lead and cadmium influences the quality of the final product. Cadmium and lead are heavy metals of unquestionable toxicity.[2] These metals are the main source of contamination for human beings found in food. In Brazil, these toxic substances are controlled in products of animal origin by the Brazilian National Residue Control Plan of the Ministry of Agriculture[3] that established maximum levels of 0.20 mg kg^{-1} Pb and 0.10 mg kg^{-1} Cd in bovine, pork, and equine meat. This program has the purpose of getting information about regional levels, making possible the identification of environmental pollution areas.[3] In this sense, the development of fast, simple, and reliable methods to monitor

the content of cadmium and lead in meat samples on a routine basis becomes indispensable.

Graphite furnace atomic absorption spectrometry (GF AAS) is one of the most sensitive techniques with limits of detection in the range from µg L^{-1} to ng L^{-1}.[4] The practical application of GF AAS for the analysis of real samples has been addressed in recent review articles.[5–7] Additionally, GF AAS allows solid and semi-solid samples to be analyzed with minimal manipulation. The book of Kurfürst[8] describes direct solid and slurry sampling analysis focusing on the characteristic methodological features of this technique up to the mid 1990s. In recent years, the direct analysis of solids and slurries by GF AAS has received much attention as an attempt to eliminate problems associated with conventional wet oxidation and dry ashing sample preparation procedures.

Of all the spectroscopic techniques, GF AAS is perhaps the most suitable one for the analysis of slurried samples. This observation stems from the intrinsic nature of the technique, which permits in-atomizer thermal transformation of the sample and, with chemical modification assistance, its simplification during the various stages of the electrothermal programs.[9,10] This methodology has the advantage of simplifying the preparation procedure, since it does usually not require special equipment and accessories, offers the benefit of reducing the possibility of analyte loss before analysis and no harsh conditions are usually needed for complete destruction of the matrix (solid or liquid). Three reviews summarize the literature about slurry sampling for

[a]*Instituto de Química, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, RS, Brazil. E-mail: mmsilva@iq.ufrgs.br; Fax: +55 51 3308-7304; Tel: +55 51 3308-6278*
[b]*Instituto Nacional de Ciência e Tecnologia do CNPq—INCT de Energia e Ambiente, Universidade Federal da Bahia, CEP 40170-115 Salvador, BA, Brazil*

direct analysis of solid material by GF AAS between 1995 and 2008: Korn et al.,[11] Piñeiro et al.,[12] and Vale et al.[13]

The sample preparation of biological tissues for direct solid sampling (DS) analyses frequently includes homogenization and lyophilization, particularly for slurry techniques,[14] where the use of finely ground material appears to be mandatory. The use of the slurry sampling for fresh meat has not been reported yet, even though it could be a good alternative for the laboratories that do not have the accessory for direct solid sampling.

An alternative for simple and fast sample pretreatment is the use of tetramethylammonium hydroxide (TMAH). TMAH is a strong base, soluble in water and in organic solvents, which under smooth heating conditions or even without heating usually completely solubilizes tissues of animal origin.[15] Moreover, the use of TMAH for slurry preparation of fresh meat should give an additional advantage because the content of water should improve the solubilization of the sample helping obtaining homogeneous slurries.

The goal of this work was to investigate a method for the determination of cadmium and lead in fresh meat by GF AAS, within the scope of the Brazilian National Residue Control Plan in products of animal origin. The homogenization of the slurry was investigated using TMAH. The use of the three-level factorial and response surface was used to optimize the slurry preparation. Emphasis was also given for comparison of the slurry technique with the conventional method of digestion adopted by the Brazilian Ministry of Agriculture.

2. Experimental

2.1. Instrumentation

All measurements were carried out using a Model AAS 5 EA atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) with deuterium background correction, equipped with a transversely heated graphite tube atomizer. NARVA hollow cathode lamps for lead and cadmium (GLE, Berlin, Germany) were used as the radiation sources with a current of 4.5 and 4.0 mA, respectively. The analytical line at 283.3 nm was used for lead determination with a spectral bandwidth of 0.5 nm, and the main analytical line at 228.8 nm was used for cadmium with a spectral bandwidth of 0.8 nm. The spectrometer was interfaced to an IBM PC/AT compatible computer. All experiments were carried out using pyrolytically coated graphite tubes with an integrated PIN platform (Analytik Jena Part No. 407-A81.025). A MPE 5 furnace autosampler (Analytik Jena AG) was used for introducing the slurry and the digested samples. Argon with a purity of 99.996% (White Martins, São Paulo, Brazil) was used as the purge gas with a flow rate of 2 L min^{-1} during all stages, except during atomization, when the flow was stopped. Integrated absorbance (peak area) was used exclusively for signal evaluation and quantification. The optimized graphite furnace temperature program is given in Table 1.

A M2P microbalance (Sartorius, Göttingen, Germany) with an accuracy of 0.001 mg was used for weighing the samples directly onto the autosampler cups. A vibratory micro-mill Model Pulverisette 0 (Fritsch, Germany), equipped with tempered steel balls, was used only to reduce the particle size of lyophilized sample (LS). This mill achieves size reduction

Table 1 Graphite furnace temperature program for determination of Pb and Cd in fresh meat samples using slurry and digestion technique

Graphite furnace program[a]	Analyte	
	Cd	Pb
Drying 1: °C; ramp/°C s^{-1}; hold/s	70; 10; 20	70; 10; 20
Drying 2: °C; ramp/°C s^{-1}; hold/s	100; 5; 60	100; 5; 60
Pyrolysis: °C; ramp/°C s^{-1}; hold/s	700; 100; 35	800; 100; 35
Atomization: °C; ramp/°C s^{-1}; hold/s	2000; 3000; 4	2000; 3000; 4
Cleaning: °C; ramp/°C s^{-1}; hold/s	2400; 1000; 4	2400; 1000; 4

[a] Purge gas (argon) flow rate: 2 L min^{-1} in all steps, except in atomization, when the gas flow was interrupted.

through the combination of impact and friction; the vibrations were controlled at amplitude of 1 mm, with time control at 1 min. An open system (digester block model 324 A 242, Quimis, Brazil), operated at a frequency of 60 Hz with a potency of 2000 W, was used for sample digestion.

2.2. Reagents and solutions

Analytical grade reagents were used throughout. The nitric acid (Merck, Germany) used in this work was further purified by sub-boiling distillation in a quartz sub-boiling still (Kürner Analy-sentechnik, Rosenheim, Germany). Distilled, deionized water with a specific resistivity of 18 MΩ cm, from a Milli-Q water purification system (Millipore, Bedford, MA, USA), was used for the preparation of samples and standards. All containers and glassware were soaked in 3 mol L^{-1} nitric acid for at least 24 hours and rinsed three times with water before use.

Lead and cadmium stock solutions (1000 mg L^{-1}) were prepared from Titrisol concentrates (Merck, Germany). The working standards were prepared by serial dilution of the stock solutions with the addition of 0.014 mol L^{-1} nitric acid (Merck, Germany) and with or without TMAH ($C_4H_{12}NOH$, 91.15 g mol^{-1}; Riedel-de Hae, Germany) 0.25% v/v. The chemical modifier investigated was a mixture of 0.05% Pd ($Pd(NO_3)_2$— Merck, Germany) + 0.03% Mg ($Mg(NO_3)_2$—Merck, Germany) + 0.05% Triton X-100 (Union Carbide, USA), all concentrations given in % m/v. The following reagents were used for sample digestion: 30% H_2O_2 (Merck, Germany) and purified nitric acid. TMAH was used for slurry preparation.

2.3. Reference materials and samples

The following certified reference materials (CRM) were used in this work for method development and validation: NIST SRM 1577b Bovine Liver and NIST SRM 8414 Bovine Muscle (National Institute of Standards and Technology, Gaithersburg, MD, USA). The lyophilized sample (LS) of bovine liver was used as a reference material for method development. This sample was previously analyzed by Nomura and Oliveira[16] using DS-GF AAS, and a value of 0.24 ± 0.02 mg kg^{-1} Pb and 0.071 ± 0.002 mg kg^{-1} Cd was obtained. This sample (LS) was ground in a vibratory mill in our laboratory. The fresh meat samples analyzed in this work, BL1, BL2 (bovine liver), PL (pig liver) and GM1 (goat muscle), were donated by the Ministry of Agriculture, Porto Alegre, Brazil. These samples were initially washed

with distilled and deionized water, cut, and homogenized using a blender (non-contaminating kitchen mixer). They were kept frozen at −10 °C in cleaned plastic bags and defrosted naturally just before the analysis.

2.4. Digestion

The reference method for digestion is recommended by the Brazilian Ministry of Agriculture (No. 400/03) for the determination of trace metals in muscle, liver and kidney by GF AAS, and was used as a comparative procedure. Around 2 g of fresh liver or muscle samples, after being ground in a blender, were weighed in triplicate directly into 50 mL glass tubes; 5 mL of concentrated nitric acid was added and heated in a digester block to 90 °C for 1 hour. The flasks were softly agitated manually to avoid foam formation. After cooling overnight, 2.0 mL of H_2O_2 was added and the mixture was heated to 90 °C for 1 hour. The digestion was completed when all fat of the meat had dissolved. After cooling, the volume was completed to 15 mL with distilled and deionized water for subsequent analysis.

The samples were analyzed at least three times by introducing 10 μL of each digested sample into the graphite tube and submitted to the temperature program (Table 1). The standard calibration technique was used, introducing 10 μL of aqueous standards. The modifier (20 μL of Pd/Mg solution described above) was introduced by the autosampler. The analyte concentration was calculated as mg kg^{-1} of the fresh substance.

2.5. Tetramethylammonium hydroxide (TMAH) slurries

The fresh meat samples or CRM (s) were weighed (around 30 mg) in triplicate, directly into the autosampler cups of the spectrometer. After, 150 μL of distilled and deionized water was added to each cup and the slurry was manually agitated with a micropipette, and then 360 μL of TMAH solution was added and the slurry was agitated again with a micropipette. Finally, the slurry was completed with 350 μL of distilled and deionized water (performing a total volume of 860 μL) and agitated again with the micropipette immediately before the analysis. Blank solution was prepared adding 360 μL of 0.25% (v/v) TMAH solution and completed with 500 μL of distilled, deionized water and agitated. The samples were analyzed at least 6 times by introducing 20 μL of slurry each. The slurries of fresh meat obtained with this method were homogeneous without need of agitation between the measurements. However, for the slurries of lyophilized sample (LS) and CRM (s), sedimentation was observed, requiring homogenization between the measurements. In this case, the slurries were agitated using a micropipette. The modifier solution (10 μL) was injected into the graphite tube using the autosampler. For all determinations calibration was performed using the standard calibration technique with aqueous standards.

The stabilization time of the slurry and of the analyte on slurry was verified. For this, consecutive measurements were accomplished using the optimized slurry composition described above. The slurries were analyzed each hour during 8 hours. Then, the slurries were kept overnight in closed autosampler cups at room temperature and in the morning the slurries were analyzed again.

The fresh meat slurries remained stable for at least 24 hours without any change in analyte concentration.

2.6. Optimization of slurry preparation

The optimization of slurry preparation was carried out using a full three-level factorial design, involving the variables: mass of sample (20–40 mg) and volume of TMAH (200–400 μL). The experimental conditions and the graphite furnace temperature program, for a sample volume of 20 μL, are given in Table 1. A full three-level factorial design was performed in order to determine the critical condition of the method, and the experiments were carried out in random order. The analytical response was integrated absorbance (peak area). The experimental data were processed using the STATISTICA 6.0.

3. Results and discussion

In order to investigate a method for the determination of cadmium and lead in fresh meat by slurry sampling-GF AAS, firstly the preparation of slurry was optimized through response surface methodology using three-level factorial design. Using this optimized composition the furnace parameters were optimized and the use of aqueous standard was investigated. After the establishment of the figures of merits, the method was applied for the determination of cadmium and lead in fresh meat and compared with the conventional method of digestion adopted by the Brazilian Ministry of Agriculture. In the sequence these steps will be discussed in details.

3.1. Optimization of the slurry preparation

As stated in the Introduction, the use of TMAH for slurry preparation offers several advantages mainly for samples of animal origin where partial or complete solubilization has been observed.[15] The slurries obtained in this work were completely homogeneous for all fresh meat samples (liver and muscle) using up to 40 mg of sample, at least 200 μL of TMAH and 500 μL of water. Apparently there was a complete solubilization of the sample as the slurries were translucent, and no particulate material was observed. The same was not observed for the LS and CRM samples where the sedimentation was observed due to remaining particulate material. But in all cases the preparation was simple and fast.

An important factor in the slurry technique is the amount of sample used in the slurry preparation. High proportions of sample are not recommended because of the difficulty of introducing the sample. On the other hand a large number of particles introduced into the furnace guarantee a low sampling error,[17,18] and increase the sensitivity. The limiting factors are difficulties in pipetting when the slurry is too concentrated and when it is too much diluted.[19] The concentration must be optimized to obtain suitable results in the analysis. The response surface methodology has been used in order to determine the optimum combination of the variables and to study their interaction on slurry analysis.[20,21] Consequently, we became interested in determining the optimum mass of sample and volume of TMAH through the response surface as well as finding the optimum zone of combination of these variables. Since the measurement of lead in meat samples is more critical than cadmium because the worse signal

Table 2 Matrix of the three-level factorial design for the optimization of slurry preparation using the lead integrated absorbance as a function of mass of BL2 meat sample (M) and volume of TMAH (V)

Experiment	M^a/mg	V^b/μL	Integrated absorbance^c/s (mean; $n = 6$)
1	40 (+)	200 (−)	0.01257
2	40 (+)	300 (0)	0.01413
3	40 (+)	400 (+)	0.01341
4	30 (0)	200 (−)	0.01511
5	30 (0)	300 (0)	0.02608
6	30 (0)	400 (+)	0.03141
7	20 (−)	200 (−)	0.01998
8	20 (−)	300 (0)	0.01642
9	20 (−)	400 (+)	0.01220

^{a} M—sample of mass. ^{b} V—volume of TMAH. ^{c} Integrated absorbance normalized for 30 mg of sample mass and 800 μL of final volume.

to noise relation observed for the former element, lead was chosen as a testing element to perform this study.

The experimental conditions used in this study were established through a preliminary atomization and pyrolysis study with slurry prepared with the maximum mass (40 mg) and minimum TMAH volume (200 μL). The measurements were performed using atomization temperature of 2000 °C and pyrolysis temperature of 800 °C.

A response surface methodology using three-level factorial design was performed, involving the following factors: mass of sample (varied from 20 to 40 mg) and volume of TMAH (200 to 400 μL). Table 2 describes the experiments performed, considering the coded and real values and the analytical signal for lead determination. The obtained relation between mass of sample (M), volume of TMAH (V) and integrated absorbance (Abs), considering the coded values, is illustrated by the equation:

$$\text{Abs} = -0.0535 + 0.0049 \times M + 3.675 \times 10^{-5} \times V - 9.415 \times 10^{-5} \times M \times M + 2.155 \times 10^{-6} \times M \times V - 1.43 \times 10^{-7} \times V \times V$$

The resolution of this equation system results in real values of sample mass of 29.25 mg and volume of 354.6 μL of TMAH. Fig. 1 shows the response surface for the lead integrated absorbance (Abs) as functions of M (mass) and V (volume). The experiment number 6 is also close to the predicted maximum. Based on these results, all of the following experiments were performed using 30 mg of sample and 360 μL of TMAH. As it will be shown later, this condition was successfully applied for the determination of cadmium in the same samples. Furthermore, the results of the stabilization test described on Section 2.5 showed that the fresh meat slurries were stable for at least 24 hours without any change in both analyte concentrations.

3.2. Temperature program and modifier

The use of chemical modifiers has become a routine in GF AAS interference-free determinations.[22] A mixture of palladium and magnesium salts was said by Schlemmer and Welz[23] to be a "more universal" modifier for GF AAS. This claim was substantiated when the modifier was shown to increase the
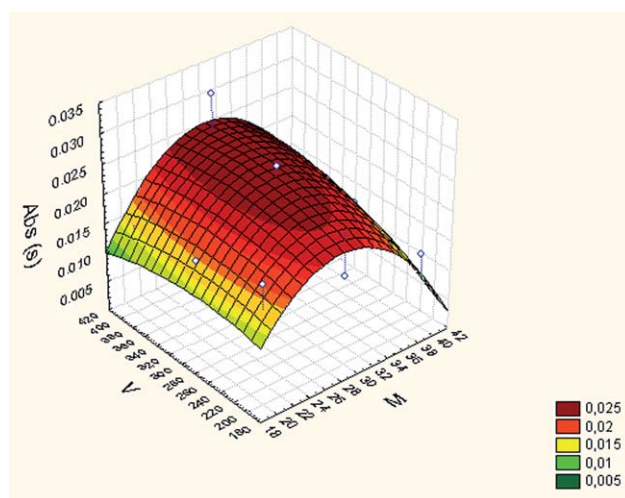

Fig. 1 Response surface plot of the lead integrated absorbance (Abs) as a function of mass of BL2 fresh meat sample (M) and volume of TMAH (V) for optimization of slurry preparation.

thermal stability of 21 elements.[24] Since then, there have been numerous reports about the effectiveness of this modifier. In practice, a mixture of palladium and magnesium has been shown to be an effective modifier for the determination of lead and cadmium in samples and reference materials of meat and fish slurries,[16,25–30] the extent of its stabilization is highly reproducible. In this work, the Pd/Mg modifier has been applied with the addition of Triton X-100 as dispersing agent, to improve the contact between sample and modifier.

The bovine liver (BL2) slurry with the composition optimized before was used to establish the furnace parameters, i.e., the optimum pyrolysis and atomization temperatures. Before that, however, it was necessary to optimize the drying stage with slurried fresh meat samples. In order to achieve a smooth and complete removal of all the water from the slurried sample, it was necessary to apply a two-step drying program with a final temperature of 100 °C, slow ramp rates and long holding times, as shown in Table 1. Fig. 2a shows the pyrolysis curves for lead aqueous standard (1 ng) in 0.014 mol L^{-1} HNO$_3$ solution and in fresh bovine liver (BL2) TMAH slurry with modifier. For the bovine liver (BL2) slurry reliable measurement of analyte signals was only possible with pyrolysis temperatures higher than 500 °C due to excessively high background absorption at lower
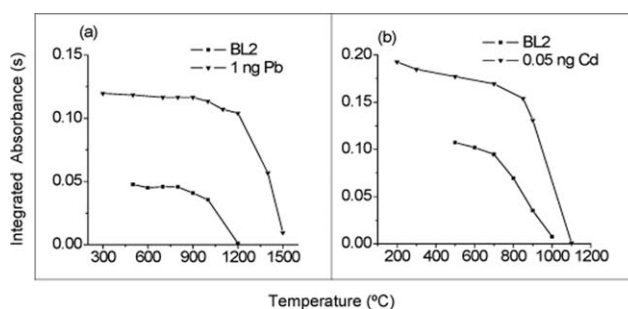

Fig. 2 Pyrolysis curves for lead (a) and cadmium (b) in aqueous solution and in fresh meat sample (BL2) slurries with Pd/Mg as chemical modifier.

temperatures as the matrix could not be eliminated efficiently. This phenomenon was observed in our group's previous work on cadmium and lead determination in fresh meat samples by DS-GF AAS.[31] A pyrolysis temperature as high as possible was chosen in order to remove or to minimize the matrix effects and background signals efficiently without loss of analyte mass. Thus, 800 °C was used for all further experiments with solutions and slurries. The pyrolysis curves obtained with Pd/Mg modifier were similar to that of our previous work with fresh meat,[31] but the maximum temperature was lower than that of the former work. This difference is most likely due to the difference between the platforms and tubes used for DS and slurry sampling.

The pyrolysis curves for cadmium in aqueous standard and in the slurried fresh bovine liver (BL2) with modifier are shown in Fig. 2b. The slurries were prepared as described above for lead. Similar to lead pyrolysis, temperatures lower than 500 °C could not be used and the maximum obtained temperatures were lower than that observed previously for fresh meat in DS.[31] For all further experiments a pyrolysis temperature of 700 °C was used.

Peak shapes and background absorption were also considered when choosing the proper furnace conditions for lead and cadmium atomization; some typical atomization and background signals are shown in Fig. 3. This figure also shows the similarity of the atomization pulses for lead obtained from an aqueous standard and fresh meat slurry with TMAH as well as the lower background signal observed for cadmium in the slurry.
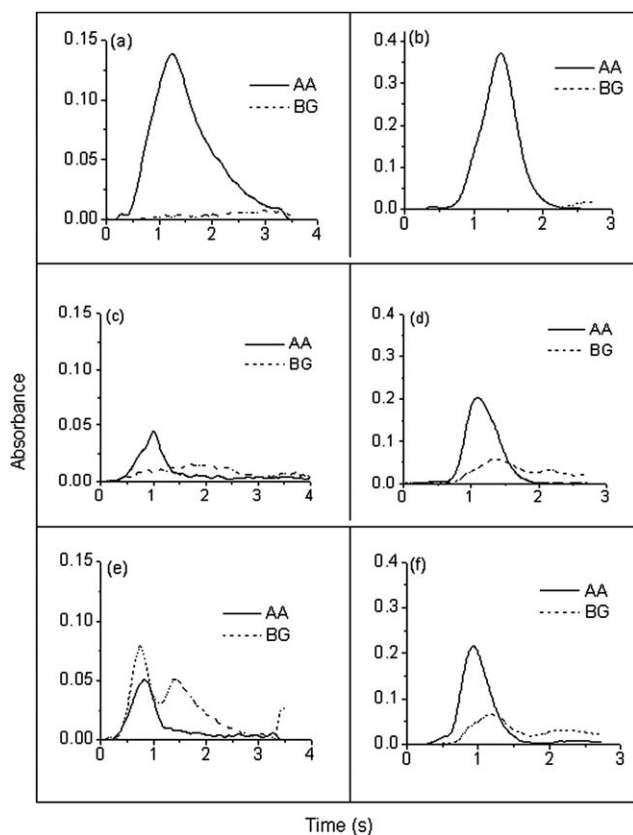

Fig. 3 Absorbance signals for lead (a, c and e) and cadmium (b, d and f) in aqueous standard: 1 ng for Pb (a) and 0.05 ng Cd (b); slurried reference material SRM1577 (c and d); slurried fresh meat sample BL2 (e and f). Chemical modifier: 0.05% Pd + 0.03% Mg + 0.05% Triton X-100.

An atomization temperature of 2000 °C was chosen based on the obtained sensitivity and peak shape for both analytes, which is in agreement with literature data for a transversely heated atomizer.[4] The complete furnace temperature program is shown in Table 1.

3.3. Figures of merit

If the main goal is to obtain results rapidly, it is essential to establish the minimum number of measurements that guarantee a final result with the required quality. In our previous work with DS-GF AAS,[31] the amount of fresh meat sample used for analysis was usually very small (0.150–10.00 mg) and during the weigh it was observed a variation of mass resulted from water loss from fresh meat. The strategy we used to weigh such samples was to record the first weight. However, in the slurry analysis this variation of mass was not observed, probably due to the higher mass used.

The relative standard deviation (RSD) of the measurements was determined for three samples with different characteristics and analyte content. The medium RSD of around 12% was obtained for lead, for six measurements of each sample. This relatively high RSD could be attributed to the higher background signal observed compared to the low analyte signal for these samples. For cadmium the medium RSD found was around 4% ($n = 6$), for the opposite reason. Considering the complexity of a biological matrix and the use of fresh meat samples the RSD found in this work should be considered acceptable.

Calibration curves were established using blank and five calibration solutions in the concentration range of 20–100 µg L^{-1} Pb (0.4–2 ng Pb) and 1.0–8.0 µg L^{-1} Cd (0.02–0.16 ng Cd) using the conditions mentioned above. Calibration using aqueous standards was checked against results obtained for CRM as will be shown in Section 3.4. As can be seen in Table 3, the slope of the regression curves for lead and cadmium, obtained using the calibration curve prepared in 0.014 mol L^{-1} HNO$_3$ or with TMAH, was very similar, indicating that aqueous standards in 0.014 mol L^{-1} HNO$_3$ could be used for calibration purposes.

The correlation coefficient (R), the characteristic mass (m_0), limits of detection (LOD) and quantification (LOQ) defined as the analyte concentration corresponding to an integrated absorbance signal equal to three times and ten times the standard deviation of the blank, respectively, obtained for lead and cadmium are also shown in Table 3. The characteristic mass values, LOD and LOQ are in agreement with literature values for both analytes,[32,33] and lower than the maximum levels of 0.20 and 0.10 mg kg^{-1} for lead and cadmium, respectively, in bovine, pig, and equine meat established by the Brazilian regulations.[3]

3.4. Lead and cadmium determination in fresh meat samples

In order to investigate the use of aqueous standards for calibration, lead and cadmium were determined in two CRM, bovine liver (NIST SRM 1577b) and bovine muscle (NBS SRM 8414) and in a lyophilized and ground sample of bovine liver (LS) previously analyzed by DS-GF AAS.[16] The results using the previously optimized slurry preparation for the CRMs are summarized in Table 4. For lead, the results have not shown significant difference at the 95% confidence level when a paired

Table 3 Analytical figures of merit for the determination of Pb and Cd by GF AAS

Analyte	Linear regression equation	R^2	m_0/pg	LOD[a] ($n = 10$)/μg kg⁻¹	LOQ[a] ($n = 10$)/μg kg⁻¹	RSD[b] ($n = 6$) (%)
Pb	$A = 0.00222 + 0.1655m$[c]	0.9956	28	24.1	80.4	12
	$A = 0.00568 + 0.1615m$[d]	0.9944	—	—	—	
Cd	$A = 0.0141 + 2.1463m$[c]	0.9991	2.0	3.84	12.8	4
	$A = 0.0234 + 2.2626m$[d]	0.9955	—	—	—	

[a] Based on slurry of 30 mg of fresh meat; volume of 860 μL. [b] As a medium of the RSD of all fresh meat samples as a slurry. [c] Calibration curve with aqueous standard in 0.014 mol L⁻¹ HNO₃. [d] Calibration curve with aqueous standard and TMAH.

Table 4 Determination of Pb and Cd in meat reference materials by slurry sampling GF AAS and calibration against aqueous standards ($n = 6$)

Sample	Pb concentration/mg kg⁻¹		Cd concentration/mg kg⁻¹	
	Certified value	Found	Certified value	Found
SRM 8414	0.38 ± 0.24	0.31 ± 0.04	0.013 ± 0.011	0.012 ± 0.002
SRM 1577b	0.129 ± 0.04	0.136 ± 0.02	0.50 ± 0.03	0.39 ± 0.02
LS	0.240 ± 0.018[a]	0.237 ± 0.036	0.071 ± 0.002[a]	0.063 ± 0.005

[a] Not certified value (by Nomura and Oliveira[16]).

Table 5 Analytical results obtained for Pb and Cd in slurried and digested fresh meat samples and calibration against aqueous standard

Sample	Concentration of Pb/mg kg⁻¹ FS[a] (mean $\pm$ SD)		Concentration of Cd/mg kg⁻¹ FS[a] (mean $\pm$ SD)	
	Slurry ($n = 6$)	Digestion ($n = 3$)	Slurry ($n = 6$)	Digestion ($n = 3$)
BL1	0.100 ± 0.016	0.086 ± 0.002	0.024 ± 0.002	0.018 ± 0.001
BL2	0.091 ± 0.009	0.105 ± 0.001	0.036 ± 0.001	0.040 ± 0.001
PL	0.074 ± 0.008	0.055 ± 0.002	0.080 ± 0.001	0.093 ± 0.005
GM1	<0.024	<0.018	0.225 ± 0.005	0.166 ± 0.003

[a] Based on wet weight (mg per kg of the fresh substance).

t-test was applied for the dataset of the obtained results. The same statistical result was obtained for cadmium. The good agreement between the results supports that aqueous calibration standards can be used for the determination of cadmium and lead in meat samples with the proposed technique.

In order to obtain information on the accuracy of the results for fresh meat slurry, a comparison of methods was performed with the digestion method adopted by the Brazilian Ministry of Agriculture for analysis of meat samples by conventional GF AAS. The results obtained for cadmium and lead in the fresh meat samples are summarized in Table 5. With the exception of cadmium in the GM sample, all samples presented concentrations below the maximum levels of lead and cadmium established by the National Residue Control Plan in products of animal origin of the Brazilian Ministry of Agriculture.[3]

The major advantages of the sample preparation of fresh meat with TMAH are its speed and a complete solubilization of the investigated samples. The slurries obtained with this method were homogeneous and give satisfactory results for fresh meat. However, for the CRM, as described in the Experimental part, it was necessary to agitate the slurry between each measurement. The paired t-test was used for the data shown in Table 5, and the results obtained for digestion and slurry methods for cadmium and lead were not significantly different at the 95% confidence

level. The overall result was very satisfactory for routine application, and good agreement was observed comparing the proposed method using slurried fresh meat and the standard method, showing that the proposed method could be used for that purpose. Even with the presence of water and fat, the slurries from the different fresh meat samples (muscle and liver) showed satisfactory results and consistent with the digestion method which eliminates all organic matter. It also should be pointed out that the lifetime of the graphite tube (around 450 heating cycles) was not greatly affected by the sample composition in the slurry.

Regarding the application of this method for other elements or samples, as showed by Nóbrega et al.[15] TMAH can be applied for partial or complete solubilization of several types of organic samples and for determination of different analytes. Additionally, in this work it was shown that the slurry of fresh meat was more homogeneous than the slurries of dried samples, given the advantage of not needing agitation between measurements. Combining these features with the high tolerance of graphite furnace to the organic matrix, it should be expected that the application for other elements and samples it is worthy of exploration. The limitation could be the range of concentration, once the method was proposed for a small amount of sample (30 mg) diluted in 860 μL, which results in a relatively low sensitivity, compared with DS-GF AAS. In the condition used in

this work, the range of concentration is around 0.06 to 3 µg kg^{-1} for lead and 0.01 to 0.5 µg kg^{-1} for cadmium. It should be emphasized that for slurries with higher concentrations, dilutions can be accomplished.

4. Conclusion

The analysis of fresh meat samples by the proposed method, in addition to using small amounts of sample and reagent, makes sample preparation very easy and avoids steps potentially prone to contamination. The use of the response surface method to optimize the slurry preparation has shown that it was possible to find the optimum zone in the experimental field of the variables under study. The positive results obtained in this work suggest that this composition can be successfully used in multielement determinations.

Finally, the analysis of slurried fresh meat by GF AAS can be applied as a rapid screening procedure within the scope of the Brazilian National Residue Control Plan in products of animal origin, helping the implementation and maintenance of sanitary controls and also in the identification of environmental pollution areas.

Acknowledgements

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