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Spectrochimica Acta Part B 62 (2007) 1037–1045

SPECTROCHIMICA ACTA PART B

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Feasibility of using direct determination of cadmium and lead in fresh meat by electrothermal atomic absorption spectrometry for screening purposes ☆

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Received 30 January 2007; accepted 24 May 2007
Available online 31 May 2007

Abstract

A method for the direct determination of cadmium and lead in fresh meat for screening purposes is proposed using electrothermal atomic absorption spectrometry. The fresh meat samples were homogenized, weighed directly onto solid sampling platforms and introduced into a transversely heated solid sampling graphite tube. The main challenges associated with this procedure, such as weighing errors and optimization of the temperature program were investigated in detail. Calibration was performed against aqueous standards and two modifiers were investigated: 0.05% Pd+0.03% Mg+0.05% Triton X-100 and 0.01% Pd+10% NH_4NO_3+0.05% Triton X-100. The former one is recommended due to the higher pyrolysis temperature obtained for cadmium and the better limits of detection of 1.9 μg kg^{-1} for lead and 0.13 μg kg^{-1} for cadmium, based on 10 mg of sample mass. 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. In order to evaluate weighing errors the fresh samples were dried (at 60 °C) to constant weight; the results obtained with fresh and dried samples were in agreement, taking the loss of weight into consideration for the latter ones. The average relative standard deviation of 14% is in concordance with the results of others using fresh meat. Comparison with the digestion method adopted by the Brazilian Ministry of Agriculture shows no significant differences between the results at the 95% confidence level. This study shows that direct analysis of fresh meet can be applied as a rapid routine screening procedure for residue control in products of animal origin, helping the implementation and maintenance of sanitary control.

Keywords: Lead; Cadmium; Fresh meat; Screening analysis; Electrothermal atomic absorption spectrometry

1. Introduction

Since 2004 Brazil is the world's largest beef and poultry and fourth-largest pork exporter. In spite of a new outbreak of foot-and-mouth disease in 2005 that led several countries to ban meat imports from regions affected by the disease, Brazil remained a significant player in world meat markets. Future increase in meat exports and greater access to global markets will depend on the success of current efforts to eliminate this disease and to implement and maintain strict sanitary controls [1]. In this sense the development of fast, reliable and inexpensive screening methods of analysis is of great importance in order to increase the number of samples that can be analyzed on a routine basis and to provide results within the shortest time possible.

The presence of heavy metals in animal tissues and meat products may result from natural occurrence in the soil, from where they are taken up by the plants that feed the animals, or due to contamination from anthropogenic sources. Several trace metals such as arsenic, cadmium, lead and mercury, are among the toxic substances controlled by the Brazilian Ministry of Agriculture. The control of these residues in meat within the Brazilian program of residue control in products of animal origin has the purpose of getting information about regional levels, making possible the identification of areas of environmental pollution [2].

☆ This paper was presented at the 9th Rio Symposium on Atomic Spectrometry, held in Barquisimeto, Venezuela, 5–10 November 2006, and is published in the special issue of Spectrochimica Acta Part B, dedicated to that conference.

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doi:10.1016/j.sab.2007.05.007

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. Lead is of public health concern because of several hazardous effects that it might cause to humans. Lead poisoning can affect many systems of the body and high level of intoxication may result in attacks of abdominal pain until coma and death [3]. Cadmium is carcinogenic and one of the most toxic metals. It might cause renal and pulmonary dysfunction, bone and liver damage. In Brazil, the program of residue control in products of animal origin of the Ministry of Agriculture [2] established maximum levels of 0.20 mg kg^{-1} Pb and 0.10 mg kg^{-1} Cd in bovine, pork, and equine meat.

The usually low content of lead and cadmium in biological materials requires the use of highly sensitive techniques for its determination. Electrothermal atomic absorption spectrometry (ET AAS) is one of the most sensitive techniques with limits of detection in the range from μg L^{-1} to ng L^{-1}, it requires only low sample volumes, tolerates high matrix concentrations and is therefore often chosen for such determinations [4]. The practical application of ET AAS for the analysis of real samples has been addressed in a recent review article [5]. Most of the methods for the determination of trace elements in solid samples require a previous digestion, which involves acid treatment in open or closed vessels [6,7]. Taylor et al. [8] published an update on applications of atomic spectrometric techniques to the determination of minor, trace and ultra-trace elements in clinical and biological materials. Sneddon et al. [9] gave an overview over preparation of solid samples for metal determination by atomic spectroscopy, including selected recent applications. However, many of these procedures are tedious and time consuming, and might be the source of systematic errors if they are not carried out by experienced personnel. A simple alternative to avoid these potential problems is the direct analysis of solid samples.

Direct solid sampling (SS) ET AAS has been shown to provide the best limits of detection because of the absence of any dilution and a minimal risk of contamination. The absence of any major sample handling makes SS-ET AAS ideally suited for fast screening analyses and routine applications. The book of Kurfürst [10] describes direct solid and slurry sampling analysis focusing on the characteristic methodological features of this technique up to the mid 1990s. A critical review of Vale et al. [11] summarizes the literature about SS-ET AAS between 1995 and 2005, so that only the most important aspects and recent development concerning the subject of this work have been considered here.

The sample preparation for SS analyses of biological tissues frequently includes homogenization and lyophilization, particularly for slurry techniques [12], where the use of finely ground material appears to be mandatory. Nomura et al. [13,14] found for bovine liver sample that both the drying and the grinding process had an influence on micro-homogeneity, which in addition was different for different elements. Homogenization of a sample might reduce heterogeneity but at the same time it increases the risk of contamination and it obviously decreases the sample throughput.

Lücker et al. published a series of papers using direct SS-ET AAS with Zeeman-effect background correction, showing that trace element distribution in animal tissues is nearly homogeneous, except in special cases, such as in muscle tissue contaminated by gun-shot residues [15]. This technique was applied to liver tissue [16,17], renal tissues [18], and Cd in equine muscle [19]. Lücker and Schuierer [20] investigated the sources of error in direct SS-ET AAS analysis of fresh animal tissue for screening purposes. They developed an automated solid sampling system and investigated the sampling of different mass of untreated animal tissue at different temperatures.

As the equipment of the above authors has been withdrawn from the market more than a decade ago, it appears more than justified to re-evaluate some of their findings using currently available instrumentation. This re-evaluation appears to be important particularly as the graphite tube used by Lücker et al. was much bigger and allowed the introduction of much greater sample aliquots than the system used in this work. Another significant difference is that the instrument used by Lücker et al. was equipped with Zeeman-effect background correction with the magnet at the lamp (direct Zeeman-effect), whereas the instrument used in this work was equipped with the cheaper, but less efficient deuterium background correction. On the other hand, the graphite tube atomizer used in this work was transversally heated and hence isothermal, and should provide more interference-free determination for elements such as Cd and Pb than the non-isothermal atomizer used by Lücker et al.

The goal of this work was to investigate the use of SS-ET AAS techniques for an accurate and rapid determination of lead and cadmium in fresh meat within the scope of the Brazilian program of residue control in products of animal origin. The main difficulties associated with this procedure, such as weighing errors, optimization of the temperature program and the use of chemical modifiers were investigated in detail. Emphasis was also given to comparison with conventional methods of digestion and solid sampling of dried and ground samples.

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 solid sampling platforms (Analytik Jena Part No. 407-152.023) and solid sampling tubes without a dosing hole (Analytik Jena Part No. 07-8130325). An M2P microbalance (Sartorius, Göttingen, Germany) with an accuracy of 0.001 mg was used for weighing the samples directly onto the SS platforms. The accurate sample mass, typically between 0.1 and 10 mg, was automatically

transmitted to the instrument computer to calculate the 'normalized integrated absorbance' (integrated absorbance calculated for 1 mg of sample) after each measurement. This normalized integrated absorbance is commonly used in SS-ET AAS to compare signals, as it is practically impossible to introduce a sample mass of exactly 1.00 mg, or always to introduce exactly the same sample mass in a series of measurements. An SSA 5 manual solid sampling accessory (Analytik Jena) was installed in the sampling area of the spectrometer, and the pre-adjusted pair of tweezers, which is part of the SSA 5, was used to transfer the SS platforms to the atomizer. An MPE 5 furnace autosampler (Analytik Jena) was used for introduction of digested solutions. 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 optimum parameters for the graphite furnace temperature program are given in Table 1. The standard calibration technique with aqueous standards was used throughout.

An oven (De Leo, Brazil) was used for sample drying. A vibratory micro-mill Model Pulverisette 0 (Fritsch, Germany), equipped with tempered steel balls, was used to reduce the particle size of dried samples. This mill achieves size reduction through the combination of impact and friction; the vibrations were controlled at an 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

Analytical grade reagents were used throughout. The nitric acid (Merck, Germany) used in this work was further purified by subboiling distillation in a quartz subboiling still (Kürner Analysentechnik, 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 h and rinsed three times with water before use.

Lead and cadmium stock solutions (1000 mg L^{-1}) were prepared from Titrisol concentrates (Merck). 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). Two different chemical modifiers were investigated: 0.05% Pd + 0.03% Mg + 0.05% Triton X-100 and 0.01% Pd + 10% NH_4NO_3 + 0.05% Triton X-100 (Pd, Mg as the nitrates and NH_4NO_3 from Merck, Germany, and Triton X-100 from Union Carbide; all concentrations in % m/v). The following reagents were used for sample digestion: 30% H_2O_2 and purified HNO_3 (both from Merck, Germany).

Table 1
Graphite furnace temperature program for the determination of Pb and Cd in meat samples by ET AAS using solid sampling (SS) and digestion technique

Graphite furnace program [a]	Technique	
	SS	Digestion
Drying 1: °C; ramp/°C s^{-1}; hold/s	90; 15; 20	70; 10; 20
Drying 2: °C; ramp/°C s^{-1}; hold/s	150; 15; 20	100; 5; 60
Drying 3: °C; ramp/°C s^{-1}; hold/s	300; 15; 30	–
Pyrolysis: °C; ramp/°C s^{-1}; hold/s	900 [b,c] or 1000 [d]; 100; 35	900 [b] or 1000 [d]; 100; 35
Atomization: °C; ramp/°C s^{-1}; hold/s	2000; FP [e]; 4	2000; FP [e]; 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.
[b] Pb: Pd + NH_4NO_3 + Triton X-100.
[c] Cd: Pd + $Mg(NO_3)_2$ + Triton X-100.
[d] Pb: Pd + $Mg(NO_3)_2$ + Triton X-100.
[e] FP = full power.

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 SS-ET AAS, and a value of 0.24 ± 0.02 mg kg^{-1} Pb and 0.071 ± 0.002 mg kg^{-1} Cd was obtained [14]. This sample was ground in a vibratory mill in our laboratory. The liver and muscle samples analyzed in this work, BL1, BL2 (bovine liver), PL (pig liver) and GM (goat muscle) were donated by the Ministry of Agriculture, Porto Alegre, Brazil. These samples were initially washed with Milli-Q water, cut, and homogenized using a blender (non-contaminating kitchen mixer). They were analyzed immediately or frozen at −10 °C in cleaned plastic bags and defrosted naturally just before the analysis.

2.4. Digestion

The reference method for digestion was the one recommended by the Brazilian Ministry of Agriculture (No. 400/03) for the determination of trace metals in muscle, liver and kidney. Around 2 g of fresh liver or muscle samples, after being ground in a blender, was weighed in triplicates 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 h. 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 heated to 90 °C for 1 h. The digestion was complete when all fat of the meat had dissolved. After cooling, the volume was completed to 15 mL with 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 submitting to the temperature program (Table 1). The standard calibration technique was used, introducing 10 μL of aqueous standards. For measurement of the digested samples, the modifiers (20 μL) were introduced by the autosampler. The analyte concentration was calculated as mg kg^{-1} of the fresh meat.

2.5. Direct analysis of fresh meat samples

Because of the continuously decreasing mass values for fresh meat samples due to water evaporation, the strategy used to weigh the samples was to keep the first weight, disregarding the loss of weight with time. Aliquots between 0.1 and 10 mg, depending on the analyte concentration, were weighed directly onto the solid sampling platforms and transferred to the graphite furnace as described above. Each sample was weighed and analyzed at least 10 times.

2.6. CRM, dried and lyophilized samples

In order to evaluate the weighing error, around 1 g of fresh meat samples was dried in an oven at 60 °C to constant weight to eliminate the humidity. All samples were dried in triplicate, ground in a vibratory micro-mill and kept in a desiccator. The grinding tube and ball were cleaned after each use to avoid cross-contamination. The CRM were analyzed as provided and the lyophilized sample was ground in the vibratory micro-mill, as described above for dried meat.

For sample masses up to 3 mg the modifier solution was introduced with a micro-pipette on top of the sample in the graphite furnace, and the temperature program was executed. For sample masses greater than 3 mg, which had to be applied for lead determination for some samples, spattering was observed during the temperature program, probably due to the high amount of water. Because of this it was necessary to inject the modifier onto the platform first and to dry it using the first three program stages of the temperature program shown in Table 1. Then the program was stopped, and the furnace allowed to cool before the sample was weighed and the entire temperature program executed. For all determinations calibration was performed using the standard calibration technique with aqueous standards.

3. Results and discussion

3.1. Wavelength selection

As the precision obtained with SS-ET AAS, among other parameters, depends critically on the sample mass introduced into the graphite tube, it is important to select an analytical wavelength that makes it possible to obtain absorbance signals within the optimum working range of the spectrometer, approximately 10–200 times the characteristic mass (m_0), with a sample mass ideally around 0.5–1.5 mg [21]. The most sensitive analytical line for lead at 217.0 nm was therefore initially investigated with the bovine liver and bovine muscle CRM. However, a very severe spectral interference was observed that could not be eliminated with the deuterium background correction system of our instrument, so that we could not use this line for our determinations. Borges et al. [22], using high-resolution continuum source ET AAS for the determination of lead in biological materials, observed strong background absorption due to the electron excitation spectrum of the phosphorus monoxide molecule PO in the vicinity of the 217.0 nm line. This molecular absorption exhibits pronounced rotational fine structure, which could be fully controlled with high-resolution continuum source AAS equipment, but cannot be handled at all by deuterium background correction; the accuracy of results obtained in this case depends on the matrix as well as on the analyte concentration [23,24].

It is worth mentioning that this spectral interference could not be corrected either with the direct Zeeman-effect, i.e. with the magnet at the lamp, used in the equipment of Lücker et al., as in this case the σ-components at both sides of the analytical line are used for correction. Obviously, fine structured background changes significantly within a few picometers, so that the background at the analytical line and in its vicinity on both sides is significantly different, resulting in correction errors. The only alternate line available for lead determination was that at 283.3 nm, the sensitivity of which is approximately a factor of three lower than that of the main resonance line. Although significantly less background absorption was observed at this wavelength, which could be managed by deuterium background correction, the lower sensitivity required the introduction of relatively high sample mass of up to 10 mg, which resulted in fairly high imprecision of the results. Sample masses higher than 10 mg could not be measured because the background absorption reached values of $A > 1$ in this case. This behavior was observed for all samples independent of the pretreatment, i.e. dried, lyophilized or fresh samples. In the case of cadmium the primary analytical line at 228.8 nm could be used without problems and proved to be well suited for this determination. Due to the significantly higher sensitivity of this analyte it was possible to analyze lower sample mass and no significant spectral interference was observed when a pyrolysis temperature higher than 700 °C was used.

3.2. Temperature program and modifiers

The use of modifiers is kind of mandatory in ET AAS, at least for the determination of volatile analytes, such as cadmium and lead in complex samples in order to facilitate the removal of the matrix without loss of the analyte. This is particularly important for a screening analysis, where a false-positive result (due to contamination) is much more acceptable than a false-negative result (due to analyte loss), as in the latter case a sample that exceeds the limits might not be detected. The choice of the best chemical modifier for a certain application appears to be one of the most important tasks of ET AAS [25].

The Pd+$Mg(NO_3)_2$ modifier was proposed by Schlemmer and Welz [26] some 20 years ago and is considered a kind of a universally applicable chemical modifier [24], as it is very effective for a wide variety of samples and analytes [4,27–29]. Ortner et al. [30] summarized in a review article several modifiers and coatings for ET AAS and their mechanisms of action. In this work the Pd+$Mg(NO_3)_2$ modifier has been applied with the addition of Triton X-100, as dispersing agent, to improve the contact between sample and modifier. The addition of Triton was also essential for spreading of the modifier solution over the entire platform surface, as has been observed in previous work with the same equipment [31], when the modifier had to be injected onto the platform before the sample (see Section 2.6). Palladium+NH_4NO_3

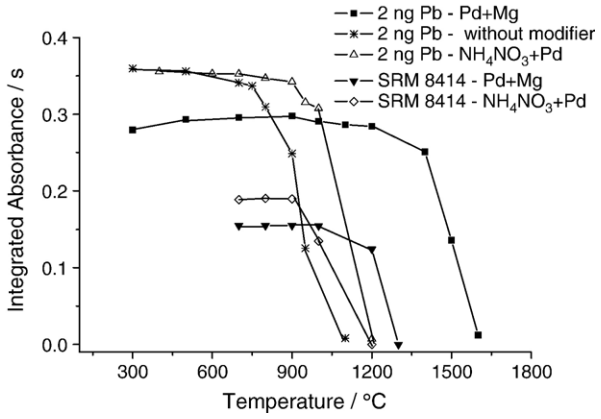

Fig. 1. Pyrolysis curves for lead in aqueous solution and in NIST SRM 8414 Bovine Muscle without modifier, with $Pd+NH_4NO_3+$Triton X-100 and $Pd+Mg(NO_3)_2+$ Triton X-100 as chemical modifiers. The data for the CRM curves are in 'normalized integrated absorbance' (integrated absorbance calculated for 1 mg of sample).

has also been proposed as a modifier for lead [4], which has been investigated as well and applied in the same way as the $Pd+Mg(NO_3)_2$ modifier, i.e., with the addition of Triton X-100.

The bovine liver and bovine muscle CRM 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 fresh meat samples. While this stage is straight-forward when aqueous solutions are used and it might even be omitted in the analysis of solid samples, it requires special attention in the case of moist samples. In order to achieve a smooth and complete removal of all the water from up to 10 mg of fresh meat, it was necessary to apply a three-step drying program with a relatively high final temperature of 300 °C, slow ramp rates and long hold times, as shown in Table 1. The pyrolysis curves for lead in the solid bovine muscle CRM and 2 ng Pb in 0.014 mol L^{-1} HNO_3 solution (also deposited on the solid sampling platform) with and without modifiers are shown in Fig. 1. For the bovine muscle CRM reliable measurement of analyte signals was only possible with pyrolysis temperatures higher than 700 °C due to excessively high background absorption at lower temperatures as the matrix could not be eliminated efficiently [23]. It can be noticed that the $Pd+Mg(NO_3)_2$ modifier provided higher pyrolysis temperatures but about 20% lower sensitivity compared with $Pd+NH_4NO_3$ for both aqueous standards and the sample, which is in agreement with the literature [4]. Pyrolysis temperatures of 900 °C and 1000 °C were chosen for the $Pd+NH_4NO_3$ and $Pd+Mg(NO_3)_2$ modifier, respectively, for all further experiments.

The pyrolysis curves for cadmium in an aqueous standard and in the solid bovine liver CRM without modifier and with the $Pd+NH_4NO_3$ and $Pd+Mg(NO_3)_2$ modifiers are shown in Fig. 2. Similar to lead a higher pyrolysis temperature and lower sensitivity were observed with the latter modifier for both aqueous standards and the CRM. As sensitivity was not a problem in the determination of cadmium, the $Pd+Mg(NO_3)_2$ modifier was chosen for all further experiments with a pyrolysis temperature of 900 °C.

Peak shapes and background absorption were also considered when choosing the proper furnace conditions for lead and cadmium; 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 with different modifiers as well as the much lower background signal observed for cadmium in the solid sample. An atomization temperature of 2000 °C was chosen on the basis of the obtained sensitivity and peak shape for both analytes and chemical modifiers in the case of lead. The complete furnace temperature program is shown in Table 1.

3.3. Sample mass and sampling errors

In solid sampling analysis a small amount of sample, typically in the range of 0.1–10 mg, is used and has to be weighed with high precision. Lücker and Schuierer [20] investigated the analytical errors resulting from water loss from fresh animal tissues before the analysis for different temperatures. Following a stabilization period, mass losses appeared to be nearly linear and independent of the input mass up to 20 mg. By means of a function using stabilization time and mass losses a 10% increase in analyte content was calculated as occurring at and below a sample mass of 0.06 mg. The temperature also had an influence on the error. After these studies these authors concluded that mass loss in matrices with high water content is only a minor source of error but they recommended some precautions for this kind of application, such as rapid sampling, low sample and room temperature and the use of as large a sample mass as possible, avoiding masses lower than 0.07 mg.

In this work the sampling error and the influence of the sample mass were investigated with four fresh samples of liver and muscle. Although a relatively low degree of heterogeneity was reported for cadmium and lead in animal tissues [17,18], the samples were homogenized before the analysis as described in the Experimental section. Immediately after the homogenization a portion of the sample was transferred to a cleaned small bag, all air was removed from the bag and it was tightly closed in order to

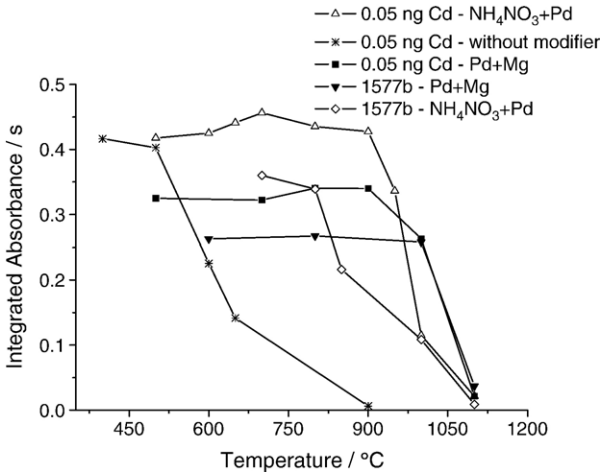

Fig. 2. Pyrolysis curves for cadmium in aqueous solution and in NIST SRM 1577b Bovine Liver without and with $Pd+NH_4NO_3+$Triton X-100 and $Pd+Mg(NO_3)_2+$Triton X-100 as chemical modifiers. The data for CRM curves are in 'normalized integrated absorbance' (integrated absorbance calculated for 0.1 mg of sample).

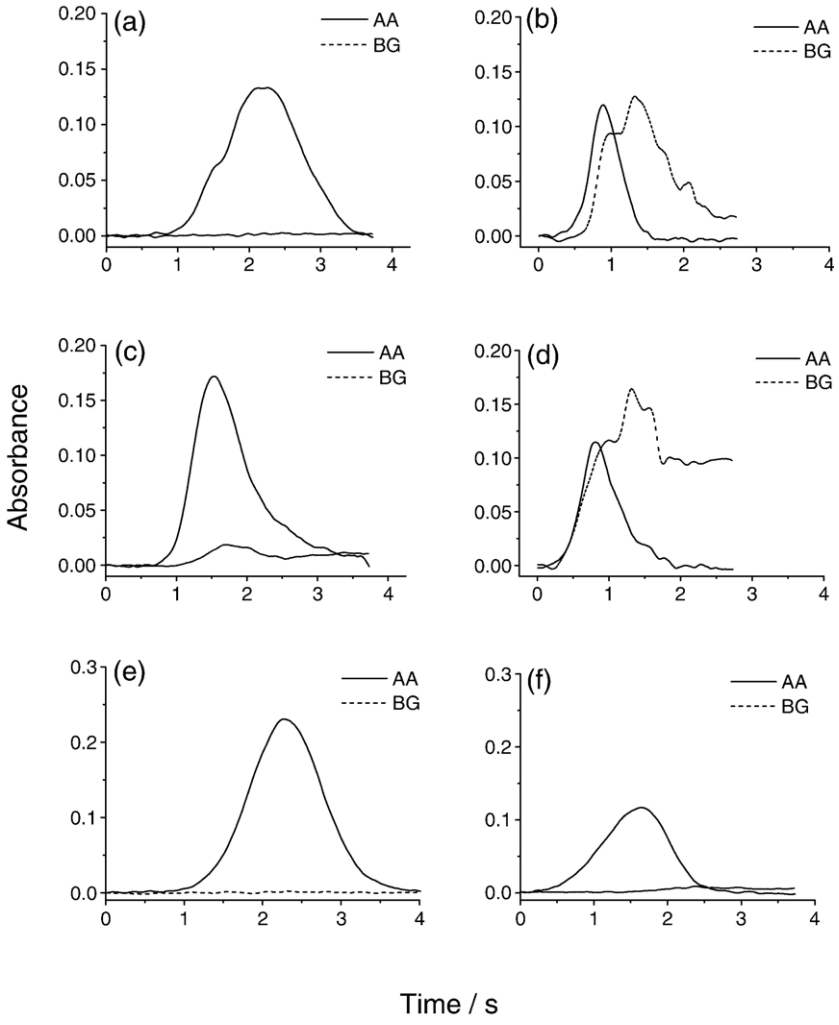

Fig. 3. Absorbance signals for lead and cadmium in aqueous standards and in NIST SRM 1577b Bovine Liver; (a, c): 2 ng Pb; (e): 0.05 ng Cd; (b, d, f): NIST SRM1577; (b,d): Pb; (f): Cd; (a, b): modifier Pd+NH_4NO_3+Triton X-100; (c, d, e, f): modifier Pd+$Mg(NO_3)_2$+Triton X-100.

avoid loss of humidity. The bag was opened only in the moment of weighing for analysis by ET AAS. This procedure reduced the risk of losses of humidity before weighing. Homogenization was considered appropriate for the present investigation in order to exclude phenomena such as the 'nugget effect' described by Kurfürst [10]. Obviously, homogenization is not necessary for screening analysis, and it might even be counter-productive as it would not allow detecting contamination due to sampling or storage.

In order to evaluate the weighing errors, part of the fresh samples was dried at constant weight to eliminate the humidity. The fresh meat samples investigated were found to contain 27± 2% of humidity. The concentration of lead and cadmium for the dried samples was corrected for this loss of weight in order to facilitate comparison with the values found when the fresh samples were analyzed. In agreement with Lücker and Schuierer [20] a small drop in weight with time was observed due to evaporation, when fresh samples were weighed. However, keeping the sample in the refrigerator around 4 °C and in the laboratory around 20–25 °C, this effect could be minimized. The strategy we used to weigh such samples was to record the first weight, not considering the weight loss with time, as proposed by the former authors.

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. For screening purposes, no more than five measurements are needed [32]. Table 2 shows

Table 2
Relative standard deviation for Pb and Cd in meat samples using different modifiers; all modifier mixtures also contained Triton X-100

Analyte	Sample	Mass range/mg	Fresh RSD/% (n=10)		Dried RSD/% (n=6)	
			Pd+Mg $(NO_3)_2$	Pd+ NH_4NO_3	Pd+Mg $(NO_3)_2$	Pd+ NH_4NO_3
Pb	BL1	2.2–2.6	12	8.4	9.1	13
	BL2	2.2–2.8	10	9.3	7.7	11
	PL	3.3–9.3	23	19	20	17
Cd	BL1	0.37–2.0	15	–	11	–
	BL2	0.85–1.8	7.1	–	7.1	–
	PL	0.30–1.3	15	–	8.1	–
	GM	0.15–0.42	17	–	4.3	–

Table 3
Analytical figures of merit for the determination of Pb and Cd by ET AAS; all modifier mixtures also contained Triton X-100

Analyte	Modifier	Linear regression equation	R	m_0/pg	LOD [a] (n=10)/μg kg^{-1}	LOQ [a] (n=10)/μg kg^{-1}
Pb	Pd+Mg$(NO_3)_2$	A=0.0205+0.325 m	0.9983	10	1.9	6.2
	Pd+NH_4NO_3	A=0.0433+0.340 m	0.9981	11	2.6	8.6
Cd	Pd+Mg$(NO_3)_2$	A=0.0317+7.406 m	0.9937	0.5	0.13	0.45

[a] Based on 'zero mass response' technique and calculated for the 10 mg of sample.

Table 5
Analytical results obtained for Pb in fresh, dried, digested meat using different modifiers; all modifier mixtures also contained Triton X-100

Sample	Modifiers	Concentration of Pb/mg kg^{-1} FS [a] (mean±SD)		
		Fresh (n=10)	Dried (n=6)	Digested (n=3)
BL1	Pd+Mg$(NO_3)_2$	0.10±0.01	0.08±0.01	0.09±0.002
	Pd+NH_4NO_3	0.07±0.01	0.07±0.01	0.09±0.001
BL2	Pd+Mg$(NO_3)_2$	0.11±0.01	0.13±0.01	0.10±0.001
	Pd+NH_4NO_3	0.07±0.01	0.11±0.01	0.08±0.001
PL	Pd+Mg$(NO_3)_2$	0.06±0.01	0.05±0.02	0.06±0.002
	Pd+NH_4NO_3	0.06±0.01	0.06±0.01	0.06±0.001
GM	Pd+Mg$(NO_3)_2$	<0.002	<0.002	<0.02
	Pd+NH_4NO_3	<0.003	<0.003	<0.01

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

the relative standard deviation (RSD) for lead in three samples with different characteristics and analyte content. Considering the complexity of a biological matrix and the use of fresh meat samples the RSD found in this work should be considered acceptable. The RSD was obviously better for samples with higher lead content (BL1 and BL2). The higher RSD (around 20%) obtained for the sample PL could be attributed to the higher background as a significantly larger amount of sample had to be introduced due to the low analyte content in this sample. The maximum sample mass that could be measured was around 10 mg. For cadmium the highest RSD was found for GM (17%), but for the opposite reason. This sample had very high cadmium content, limiting the mass of sample that could be weighed to give a signal within the linear range. Based on the data of Table 2 it can be concluded that a better RSD was obtained when the mass range analyzed was around 1–3 mg, for both elements. The mean RSD of 14% for both cadmium and lead in fresh meat samples is in concordance with values reported by Lücker [33] and close to typical values obtained with direct solid sampling analysis of dried and ground samples. Obviously, for a screening method there is no need to detect the lowest analyte concentrations or to determine excessively high analyte concentrations with high accuracy. A screening method has to be able to detect outliers reliably, i.e. it has to work with good accuracy and reasonable precision in the vicinity of the threshold limit.

3.4. Figures of merit

Calibration has often been considered problematic in SS-ET AAS because the processes of analyte vaporization and atomization might depend on the form in which the analyte is present and might also be affected by the matrix. Calibration using one or more solid CRM, besides being expensive and therefore not recommended for routine analysis, was found to contribute significantly to imprecision, as the uncertainty of the certified value is fully introduced into the calibration. However, several studies have shown that calibration against aqueous standards is feasible in SS-ET AAS after careful program optimization [11]. Calibration curves were established using a blank and five calibration solutions in the concentration range 50–200 μg L^{-1} Pb (0.5–2 ng Pb) and 2.5–10 μg L^{-1} Cd (0.025–0.1 ng Cd) using the above-mentioned conditions. Calibration using aqueous standards was checked against results obtained for CRM as will be shown in Section 3.5. The linear regression equations, the correlation coefficient (R) and the characteristic mass (m_0) obtained for lead and cadmium are given in Table 3. The 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, are also shown in Table 3. The blank measurements were carried out according to the 'zero mass response' technique [10], introducing repeatedly an empty solid sampling platform, containing only the modifier, followed by a regular atomization cycle. The characteristic mass values, LOD and LOQ are in good agreement with literature values for both analytes [14] and comfortably 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 [2].

3.5. Analytical results

The feasibility of using calibration against aqueous standards for SS-ET AAS was investigated by determination of lead and cadmium in two CRM, bovine liver (NIST SRM 1577b) and bovine muscle (NBS SRM 8414) and in a lyophilized and

Table 4
Determination of Pb and Cd in meat reference materials using SS-ET AAS and calibration against aqueous standards (n=6); all modifier mixtures also contained Triton X-100

Sample	Pb concentration/mg kg^{-1}			Cd concentration/mg kg^{-1}	
	Certified value	Pd+Mg$(NO_3)_2$	Pd+NH_4NO_3	Certified value	Pd+Mg$(NO_3)_2$
SRM 8414	0.38±0.24	0.33±0.05	0.33±0.04	0.013±0.011	0.008±0.005
SRM 1577b	0.129±0.04	0.12±0.03	0.11±0.02	0.50±0.03	0.43±0.02
LS	0.24±0.02 [a]	0.26±0.02	0.28±0.03	0.071±0.002 [a]	0.075±0.004

[a] From Ref. [14].

Table 6
Analytical results obtained for Cd in fresh and dried meat using the Pd+Mg $(NO_3)_2$+Triton X-100 modifier

Sample	Cd concentration/mg kg^{-1} FS[a] (mean±SD)	
	Fresh (n=10)	Dried (n=6)
BL1	0.020±0.003	0.019±0.002
BL2	0.042±0.003	0.042±0.003
PL	0.072±0.011	0.086±0.007
GM	0.31±0.06	0.35±0.02

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

ground sample of bovine liver (LS) previously analyzed by SS-ET AAS [14]. The results are shown in Table 4. For lead the use of the Pd+$Mg(NO_3)_2$ modifier appears to give better results but there was no significant difference at the 95% confidence level when a paired t-test was applied for the dataset of the results obtained with each modifier related to the certified values. 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 lead and cadmium in meat samples with the proposed technique.

In order to obtain information on the accuracy of the results for fresh meat, a comparison of methods was performed. Firstly, SS-ET AAS was used to analyze the dried and ground samples as we knew that this technique is well suited for this determination, as shown by the results in Table 4 and in the literature [14]. In order to compare the results with those obtained for fresh meat samples the concentrations of lead and cadmium for the dried samples were corrected for wet weight, as described earlier. Secondly, the digestion method adopted by the Ministry of Agriculture for investigation of meat samples was also used for lead determination. The results obtained for lead and cadmium in the meat samples are summarized in Tables 5 and 6. With the exception of cadmium in the GM sample, all samples presented concentrations below the maximum levels of lead and cadmium established by the program of residue control in products of animal origin of the Brazilian Ministry of Agriculture [2].

For lead both modifiers presented good performance and the results for fresh meat compared well with those obtained for dried and digested samples. Although the Pd+NH_4NO_3 modifier was more efficient in reducing the background, the Pd+$Mg(NO_3)_2$ modifier is recommend based on the better LOD obtained, due to the lower blank value caused by this modifier. This modifier also presented better performance for the determination of cadmium. The Analysis of Variance (ANOVA) was applied to the data of Table 5 and showed that the results are not significantly different at the 95% confidence level. The paired t-test was used for the data shown in Table 6, and the same statistical result was obtained for Cd. The overall result was very satisfactory for routine application, and good agreement was observed comparing the proposed method using fresh meat and the alternate methods, showing that the proposed method could be used for the purpose.

4. Conclusion

The proposed method makes possible an accurate determination of lead and cadmium in fresh meat. This very sensitive and rapid determination, which uses aqueous standards for calibration, makes the procedure highly suitable for routine application. The mixed modifier used in this work could stabilize both analytes in fresh meat to pyrolysis temperatures of at least 900 °C, avoiding analyte losses and hence false-negative results. The homogenization of the samples used in this work is obviously not necessary for routine screening analyses as it would slow down the sample throughput significantly. Particularly important for rapid screening is the fact that the digestion procedure used for ET AAS or other solution techniques is avoided or alternately the drying and grinding pretreatment necessary for conventional SS-ET AAS. This study has shown that direct analysis of fresh meat by ET AAS can be applied as a rapid screening procedure within the scope of the Brazilian program of residue control in products of animal origin, helping the implementation and maintenance of sanitary controls. It might be assumed that this procedure could also be applied for the determination of other trace elements of interest in meat and meat products as long as their concentration is within the working range of the technique.

Acknowledgements

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – Process number 476689/2004-0), Fundação de Amparo a Pesquisa do Rio Grande do Sul (FAPERGS) and Fundação de Amparo a Pesquisa do Estado da Bahia (FAPESB) for financial support. I.C.F.D., M.M.S. and M.G.R.V. have scholarships from CNPq; B.W. has a research scholarship from FAPESB. The authors are also grateful to Analytik Jena AG for the donation of an atomic absorption spectrometer. We also thank Maria Aparecida B. Espirito Santo from the Ministry of Agriculture/Brazil for donation of samples, for supplying of digestion method and a period of training for I.C.F.D., and Cassiana S. Nomura and Pedro V. Oliveira for the donation of the lyophilized sample. The authors would also like to thank Annelise S. Hammes, Tamara S. Castilhos and Mariana Assis for their careful experimental work.

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