J. Braz. Chem. Soc., Vol. 22, No. 10, 1850-1857, 2011.
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0103 - 5053 $6.00+0.00

Article

Determination of Ca, Cu, Fe and Mg in Fresh and Processed Meat Treated with Tetramethylammonium Hydroxide by Atomic Absorption Spectrometry

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Um método simples para o tratamento de amostras de carne fresca e processada com hidróxido de tetrametilamônio (TMAH) é proposto para a determinação de Ca, Fe e Mg por espectrometria de absorção atômica com chama (FAAS) e Cu por espectrometria de absorção atômica com forno de grafite (GFAAS). A exatidão foi avaliada por comparação dos resultados usando outros dois procedimentos de preparo de amostra e por análise de materiais de referência certificados. Não houve diferença significativa entre os resultados obtidos, em um nível de confiança de 95%. Os limites de detecção para Ca, Cu, Fe e Mg foram 45,0, 0,2, 16,0 e 0,3 μg g^{-1}, respectivamente. A espectrometria de absorção atômica de alta resolução com fonte contínua em forno de grafite (HR-CS GFAAS) foi empregada para avaliar as interferências espectrais na determinação de Cu. Entretanto, nenhuma interferência foi encontrada. O método proposto é simples, rápido e confiável para análise de produtos cárneos e não requer o uso de equipamentos especiais nem de ácidos fortes no preparo das amostras.

A simple method for treating fresh and processed meat with tetramethylammonium hydroxide (TMAH) is proposed for the determination of Ca, Fe and Mg by flame atomic absorption spectrometry (FAAS) and Cu by graphite furnace atomic absorption spectrometry (GFAAS). The accuracy was evaluated by comparison of the results by using two other sample preparation procedures and by the analysis of certified reference materials. No significant differences between the results were found at the 95% confidence level. Limits of detection for Ca, Cu, Fe and Mg were 45.0, 0.2, 16.0 and 0.3 μg g^{-1}, respectively. High-resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS GFAAS) was employed to evaluate the spectral interference in the determination of Cu. However, no interference was found. The proposed method is simple, fast and reliable for meat products analysis and does not require especial equipment, neither strong acid for sample preparation.

Keywords: tetramethylammonium hydroxide, fresh and processed meat, atomic absorption spectrometry, trace metals

Introduction

There are many controversies about the intake of red meat for human consumption, with restrictions mainly focused on the content of saturated fat and cholesterol.[1-3] Thus, since the mid-nineties the consumption of red meat, mostly in developed countries, has decreased and the consumption of white meat (chicken and fish) has been adopted as a substitute.[4] However, the meats in general are of great importance in food, because they are a source of large quantities and varieties of nutrients, being rich in essential substances for growth and development of the living beings.[4,5]

With the growing demand for food ready for consumption, the market of processed meat products has

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undergone a wide expansion, since these meals can be prepared and served in a short time. In counterpart, several studies have showed gains or losses of nutrients during the processing steps (irradiation, excessive heat and freezing), causing changes in the meat composition, with the possible formation of compounds potentially harmful to human health, and affecting, therefore, their nutritional value.[6-11] Thus, the growing interest in determining trace constituents in industrialized products measuring the concentration of several essential elements such as Ca, Cu, Fe, Mg and Zn for nutritional purposes, is no surprise. The development of analytical methods for quality control processes is very important in order to monitor the chemical composition of these foods.[12-14]

The most conventional sample preparation methods used for the determination of metals in food samples using spectroanalytical methods, involve the digestion of the sample with acids that have oxidant characteristics, using a digester block or microwave heating.[15] However, many of these procedures are tedious, require complex laboratory equipment, and increase the risk of contamination and analyte loss by volatilization or adsorption in flasks. As example, the digestion of fats, proteins or amino acids is incomplete due to the low oxidation potential of nitric acid. In order to complete the digestion usually other acids (sulfuric and/or perchloric acid) are added. Simple alternatives to avoid these potential problems include the direct analysis of the solid samples or the use of slurry sampling, which significantly reduces the time required for sample preparation and the use of corrosive and hazardous reagents.[15-18]

When spectrometric techniques with conventional pneumatic nebulization of the sample are employed (as in flame atomic absorption spectrometry, FAAS), the sample has to be digested with oxidizing acids, using either hot-plate or microwave heating.[19,20] However, these traditional techniques for sample preparation are expensive, time consuming, require large amounts of reagents, which can generate hazardous waste and increase the risk of contamination of the sample with the analytes. On the other side, loss of analyte by volatilization or adsorption on the flasks walls may occur due to these procedures.

Alternative methods of sample preparation involving complete or partial solubilization of the matrix have been reported in the literature. Slurry preparation is a particularly attractive technique, since combines the advantages of liquid and solid samplings, frequently allowing the use of aqueous standards for calibration, as well as, presenting a lower susceptibility to contamination due to less manipulation of the sample and to less use of reagents. However, one aspect of this technique that should be taken into consideration is the homogeneity and stability of the slurry that depend on the particle size distribution.[15,17,21-23]

In general, biological samples are easily solubilized at room temperature with tetramethylammonium hydroxide (TMAH), not requiring the use of energy, such as microwave, ultrasonic or hot plates for heating, which prevents the loss of volatile analytes before analysis. This reagent is a strong organic base (pH 13.4 up to 14.7) with the chemical formula $(CH_3)_4NOH$, soluble in water or alcohols with the property of solubilizing different kinds of tissues, stabilizing volatile elements in the slurry for months. Besides this, the use of TMAH results in a fast, easy, simple and reproducible method for sample preparation. Due to the great simplicity of the preparation of biological samples with TMAH, many studies can be found in the literature. Since a review about alkaline solubilization was published by Nobrega *et al.*[24] in 2006, many procedures for treating biological samples with TMAH were reported as an alternative to conventional digestion for the determination of metallic elements by atomic spectrometry methods.[24-27]

This work describes the development of a fast and simple method for sample preparation of meat with TMAH for the determination of Ca, Cu, Fe and Mg by atomic absorption spectrometry. Calibration was carried out by using aqueous standards prepared in the presence of TMAH. The accuracy of the method was evaluated through a comparative study of different procedures for sample preparation, as well as, by the analysis of certified reference materials of meat. To the best of our knowledge, this procedure was not proposed before.

Experimental

Instrumentation

A Model AA-6300 atomic absorption spectrometer with flame (Shimadzu, Japan) equipped with deuterium background correction was used for the determination of Ca, Fe and Mg in commercial samples of processed meat and fresh meat (beef and pork). An air-acetylene flame was used for all determinations. The spectrometer was operated using wavelengths of 422.7, 248.3 and 285.7 nm and a spectral band pass of 0.7 nm, for determinations of Ca, Fe and Mg, respectively. The lamp current used for the respective elements was 10, 12 and 8 mA.

Due to its superior background correction capacity and to the much higher level of information provided, a high-resolution continuum source atomic absorption spectrometer (HR-CS AAS) Model Contra AA 700 equipped with a transversely heated graphite tube atomizer

and a MPE 60 autosampler (Analytik Jena AG, Jena, Germany) was used for Cu determination. A xenon short arc lamp working in optimized hot-spot mode at 300 W for the full measurement range of AAS (185-900 nm) was used as radiation source. A high-resolution double monochromator, consisting of a prism as pre-monochromator and an echelle grating monochromator, providing a spectral bandwidth *per* pixel of *ca.* 2 pm at 200 nm, was used to promote spectral dispersion of the continuum radiation and a linear charge coupled device (CCD) array detector with 588 pixels for the detection of the radiation. Argon with a purity of 99.996% was used as the purge gas with a flow rate of 2 L min^{-1} during all stages, except during atomization step, when the flow was stopped. An analytical line at 327.396 nm was employed for Cu, using integrated absorbance (peak area) for signal evaluation. The temperature program of the graphite furnace was optimized through pyrolysis and atomization curves. The adopted pyrolysis and atomization temperatures were 1200 and 2300 °C, respectively.

The samples were weighed using an Ohaus Adventurer analytical balance (Model AR 2140, Pine Brook, NJ, USA) with a resolution of 0.1 mg and tare maximum of 210 g. For the sample acid digestion, a heated digester block was used (MA-4025 from Marconi, Piracicaba, SP, Brazil). In order to facilitate the sample solubilization with formic acid, an ultrasonic bath (Model Q335D, Quimis, SP, Brazil) was used.

Reagents, samples and reference materials

For all procedures, analytical grade reagents were used throughout. The samples and standards were prepared using high-purity water with a resistivity of 18.3 MΩ cm, which was obtained from a Direct-Q 3 Water Purification System (Millipore Corporation, Bedford, MA, USA). Nitric acid (Synth, Brazil) was purified twice by sub-boiling in a MA-075 quartz system (Marconi, Piracicaba, SP, Brazil). All glassware were washed and subsequently soaked in 10% (v/v) HNO_3 for at least 48 h and then rinsed three times with ultrapure water before use. Working solutions of Ca, Cu, Fe and Mg were prepared daily by appropriate dilution of the stock solution containing 1000 mg L^{-1} (Fluka, Buchs, Germany) in ultrapure water. The following reagents were used for sample digestion: formic acid (06450, Fluka Analytical, Germany), tetramethylammonium hydroxide 25% (m/v) (331635, Sigma Aldrich, Germany), 35% (v/v) hydrogen peroxide (95299, Fluka Analytical, Germany) and nitric acid (Synth, Brazil).

For the development of the proposed procedure and verification of analyte concentrations, meat samples of beef fresh (FM1), pork fresh (FM2), as well commercial samples of canned sliced bovine meat (PM1), canned sausage (PM2) and canned meatballs (PM3) from different Brazilian manufacturers were used. These samples were initially washed with ultrapure water, cut and homogenized using a blender (non-contaminating kitchen mixer). They were analyzed immediately, in triplicate, or frozen at −16 °C in clean plastic pots and defrosted naturally just before analysis.

The following certified reference materials (CRM) were used in this work for method development and to check the accuracy of the proposed procedure: 1546 Meat Homogenate, 1577c Bovine Liver and 8414 Bovine Muscle Powder, all produced by the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

Sample treatment

The samples were prepared using three different procedures, described below. Procedures 2 and 3 were used to verify the accuracy of the proposed method using the alkaline solubilization with TMAH.

Alkaline solubilization with TMAH (Procedure 1)

The samples were prepared directly in polyethylene flasks by simply mixing approximately 500 mg of the meat sample with 750 µL of TMAH solution 25% (m/v), then the mixture was placed in an ultrasonic bath at 65 °C with the dissolution occurring in approximately 30 min. After the complete solubilization and cooling, the volume was made up to 50 mL with ultrapure water, and the final concentration of TMAH was 0.38% (m/v). The resulting sample slurry was submitted to analysis.

For determination of Cu by HR-CS GFAAS, samples were subjected to the same treatment, however weighing approximately 250 mg of the sample in the presence of 500 µL of the TMAH 25% (m/v). The volume was made up to 14 mL with ultrapure water, being 0.89% (m/v) the final concentration of TMAH.

Digestion with HNO_3 and H_2O_2 (Procedure 2)

Meat samples were treated by acid digestion based on the method described by Damin *et al.*[28] Approximately 500 mg of fresh samples were weighed in triplicates directly into 50 mL glass digester tube; 5 mL of concentrated HNO_3 was added, and the mixture was heated in a digester block at 90 ºC for 1 h. After cooling, 2 mL of H_2O_2 was added and the mixture was heated at the same temperature for an additional 1 h. The digestion was considered complete when all fat of the meat had dissolved. After cooling, the volume was completed to 50 mL with ultrapure water for subsequent analysis.

Solubilization with formic acid (Procedure 3)

Meat samples were treated with formic acid for solubilization based on the method proposed by Scriver *et al*.[17] The same mass used in the previous procedures was weighed directly in polyethylene flasks and 10 mL of formic acid was added. The mixture was placed in an ultrasonic bath at 70 °C for 4-6 h to complete the solubilization process. After cooling, the flask was filled to 50 mL with the ultrapure water for subsequent analysis.

Methodology

Initially, studies were made in order to verify sampling error and calculate the humidity content present in each sample. For this, about 1 g of fresh and processed meat samples was dried in an oven at 103 ºC up to a constant weight to eliminate the humidity. All samples were dried in triplicate and kept in desiccators until weighing.

As previously described, the meat samples were treated with TMAH, nitric acid and formic acid, for comparison purposes. The analytical results were obtained by preparation of calibration curves using solutions in the same medium used for treating the samples. The sample solutions were diluted with de-ionized water to be within the linear calibration range.

For the determination of Ca and Mg in the Air-C_2H_2 flame, it was necessary to add a buffer solutions of $LaCl_3$ 0.1% (m/v) in the samples and standards for minimizing the possible interferences from oxide formation, according to the recommendations suggested by the manufacturer. For determination of Cu, the samples were analyzed at least three times by introducing 20 µL of each sample slurry into the graphite tube and submitted to the temperature program. For sample measurement, there was no need to use modifier, since a high pyrolysis temperature was allowed. This facilitated the removal of the matrix without loss of the analyte during this stage.

Results and Discussion

Comparison of sample preparation procedures

Among the most frequently applied methods for the sample preparation in food analysis employing spectrometric techniques are the sample decomposition by acid or mixtures of acids, carried out in open tubes, vessels heated on block digester, hot plate or in closed vessels at elevated pressure with the assistance of microwave energy.[15] However, recently the use of slurry preparation, particularly with the assistance of ultrasound at room temperature, has became an increasingly attractive alternative. Also the use of formic acid in similar applications has provided good results.[17,29] As shown in this work (Table 1), similar results in the medium of TMAH were obtained by analysis of processed meats using acid digestion and formic acid solubilization. The concentrations obtained using the three different methods of sample preparation are in agreement for all the studied analytes, the values of the concentrations obtained with the respective methods did not vary more than 5%. The agreement is already a good indication of the accuracy of the proposed method.

The meat samples treated with TMAH were opaque indicating a slurry formation, requiring a light heating for complete dissolution of the samples. In the formic acid medium, the samples presented the same appearance, being necessary greater exposure in higher temperatures for complete dissolution of samples. However, the sample decompositions by acid on a heating block needed better optimization in view of the high-fat, and factors such as HNO_3 and H_2O_2 volumes and the digestion time were

Table 1. Results for Ca, Fe and Mg (in mg kg^{-1}) obtained by FAAS in processed meats (average ± standard for n = 3), using alkaline treatment (TMAH)

Analyte	Sample	TMAH			Formic acid			HNO_3		
		Found / (mg kg^{-1})	RSD / %	LOD / (µg g^{-1})	Found / (mg kg^{-1})	RSD / %	LOD / (µg g^{-1})	Found / (mg kg^{-1})	RSD / %	LOD / (µg g^{-1})
Ca	PM1	151.3 ± 2.1	1.4	45	148.97 ± 6.30	4.23	40	164.00 ± 3.96	2.44	61
	PM2	2433 ± 61	2.5		2529.23 ± 61.00	2.41		2456.80 ± 47.46	1.93	
	PM3	1549 ± 32	2.1		1579.63 ± 40.39	2.56		1581.60 ± 62.26	3.94	
Fe	PM1	172.4 ± 1.4	0.8	16	175.74 ± 2.17	1.23	24	170.40 ± 4.33	2.54	18
	PM2	50.05 ± 3.54	7.1		50.35 ± 0.78	1.55		52.45 ± 0.21	0.40	
	PM3	106.3 ± 7.8	7.3		114.10 ± 1.71	1.50		113.37 ± 4.57	4.03	
Mg	PM1	308.7 ± 7.9	2.5	0.3	307.33 ± 8.37	2.72	0.3	301.00 ± 17.53	5.82	0.3
	PM2	467.8 ± 1.5	0.3		468.57 ± 3.96	0.85		461.67 ± 5.06	1.10	
	PM3	350.63 ± 4.17	1.2		353.57 ± 3.07	0.87		357.33 ± 12.64	3.54	

PM1: sliced bovine meat; PM2: sausage; PM3: meatballs.

found to be the most important parameters. In this medium, incomplete digestion was verified and can be related to the relatively low oxidation potential of nitric acid at temperatures lower than 200 °C.[15]

In order to further evaluate the accuracy of the results obtained by the proposed method, two certified reference samples of meat were analyzed, bovine liver (NIST 1577c) and meat homogenate (NIST 1546). According to the results presented in Table 2 and the application of the *t*-test for a confidence level of 95%, a good agreement between the measured values obtained with TMAH treatment and the certified values can be observed, proving the veracity of the results.

Table 2. Analytical results for Ca, Fe and Mg by FAAS and Cu by HR-CS GFAAS obtained in different certified reference materials (average ± standard for n = 3) using alkaline treatment (TMAH)

Sample / Analyte	TMAH		
	Certified value / (mg kg^{-1})	Determined / (mg kg^{-1})	RSD / %
Bovine liver (NIST 1577C)			
Ca	131.0 ± 10.0	125.4 ± 2.6	2.1
Cu	2.84 ± 0.45	2.41 ± 0.07	2.9
Fe	197.9 ± 0.7	197.2 ± 6.8	3.4
Mg	620.0 ± 42.0	575.4 ± 1.1	0.2
Meat homogenate (NIST 1546)			
Ca	323.0 ± 28.0	325.1 ± 1.8	0.5
Cu	0.60 ± 0.04	0.62 ± 0.01	1.6
Fe	10.1 ± 0.7	10.3 ± 0.4	3.9
Mg	163.0 ± 11.0	163.8 ± 6.8	4.1

The comparison of the three studied methods reveals that the use of TMAH brings some advantages considering that the solubilization in this medium is fast (approximately 30 min with the use of ultrasonic bath) at room temperature. Moreover, small amounts of the reagent TMAH are necessary, resulting in a small dilution and small amount of residues, contributing with the green chemistry. The other methods such as solubilization in formic acid or acid digestion require heating and more preparation time (*ca.* 4-6 h for formic acid solubilization and 2 h for acid digestion). The reagent consumption in these cases is higher than with the TMAH treatment.

Copper determination

It is known that the more sensitive analytical line of Cu is overlapped by an absorption band of the PO molecule. In order to evaluate the possible spectral interference on the Cu determination at 324.754 nm in fresh and processed meats after alkaline treatment with TMAH, a high-resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS GFAAS) was used. This technique was chosen due to the much higher level of provided information when compared with conventional line-source spectrometry. However, no spectral interference was found, which can be consequence of the dilution and partial destruction of the matrix when the alkaline treatment is employed and certainly to the high pyrolysis temperature. The pyrolysis curves for Cu in all analyzed samples and in a certified reference material after alkaline treatment were very similar, indicating that the thermal stability is virtually independent on the matrix for the different meats. The used pyrolysis and atomization temperatures were 1200 and 2300 °C, respectively.

In order to verify the accuracy of the results for the analyzed meat samples, Cu was also determined in two different certified reference materials (meat homogenate and bovine muscle powder) and the results were submitted to statistical tests. According to the results (Table 2), it is possible to observe a good correlation between the obtained results for these materials after alkaline treatment and the certified values.

Figures of merit

The figures of merit for the calibration curves for all analytes (Ca, Cu, Fe and Mg) are shown in Table 3. The characteristic mass values for Cu analyte are in good agreement with those recommended by the manufacturer. Good linear correlation coefficients in the curves were obtained ($R > 0.99$), independent of the used method for sample preparation. The sensitivities, given by the slope of the curves, were also very similar in all media, as well as the limits of detection (LODs). The LOD is defined as the concentration equivalent to three times the standard deviation of ten replicate measurements of the blank divided by the slope of the calibration curve.

Analytical results

Different kind of red meats (fresh bovine, fresh pork, canned sliced bovine meat, canned sausage and canned meatballs) were treated with TMAH and analyzed by the proposed procedure. The obtained concentrations are shown in Tables 1 and 4.

Several literature data have shown that meat and meat products in general have in their composition low

Table 3. Figures of merit for the determination of Ca, Fe and Mg by FAAS and Cu by HR-CS GFAAS in meat samples using three different sample treatments

Analyte	Range / (mg L^{-1})	TMAH			Formic acid			HNO_3		
		a / (L mg^{-1})	LOD / (mg L^{-1})	R	a / (L mg^{-1})	LOD / (mg L^{-1})	R	a / (L mg^{-1})	LOD / (mg L^{-1})	R
Ca	0.5-2.0	0.0969	0.1	0.9999	0.1013	0.1	0.9991	0.0717	0.2	0.9999
Cu[a]	5-20	0.0189	0.8	0.9934	–	–	–	–	–	–
Fe	0.5-2.0	0.0819	0.05	0.9999	0.0751	0.07	0.9995	0.0826	0.05	0.9998
Mg	0.1-0.4	1.5646	0.001	0.9999	1.5702	0.001	0.9996	1.4715	0.001	0.9991

Range: concentration range of the calibration solutions; a: slope of the calibration curve; LOD: limit of detection in the measuring solution; R: correlation coefficient of the calibration curve; [a]Cu: range and LOD in μg L^{-1} and a in L μg^{-1}.

concentrations of Ca.[14,30] However, according to the results in Tables 1 and 4, relatively high levels of this element were found in sausages and meatballs sample. This can be justified from the nature of the raw material used to manufacture these meat products, once that they are mechanically separated and the obtained part from leftover meat that remains attached to the bones after removing the bones.[31,32]

For Fe, the detected concentrations in this study show that fresh meat samples have a content in the same order of magnitude in comparison to other previously published reports (Table 4).[4,17,33] On the other side, higher levels of Fe were verified in samples of processed canned meat of sliced bovine and meatballs (Table 1). This can be attributed to the internal corrosion of cans, which end up causing the increase of the metal contents. According to Tahán *et al*.,[12] the metal concentrations in canned food vary depending on the type and origin of the food, pH of the canned product, oxygen concentration in the headspace, quality of the inside lacquer coating of cans, storage, place, etc. Arvanitoyannis[6] verified that metal content increased with storage time. The results of this study for Fe, Cu and Sn indicated that content of these elements were not stabilized even after a 24 months. The level of these analytes was rather high, that may exceed legal limits when stored for a long period of time.

Table 4. Results (in mg kg^{-1}) obtained in fresh meats (average ± standard for n = 3) using alkaline treatment (TMAH) for Ca, Fe by FAAS and Cu by HR-CS GFAAS in fresh and processed meats

		TMAH	
Analyte	Sample	Found / (mg kg^{-1})	RSD / %
Ca	FM1	78.35 ± 0.07	0.1
	FM2	406.9 ± 5.8	1.4
Cu	FM1	3.22 ± 0.03	0.9
	FM2	1.34 ± 0.02	1.5
	PM1	3.67 ± 0.03	0.8
	PM2	2.37 ± 0.04	1.7
	PM3	1.39 ± 0.01	0.7
Fe	FM1	71.37 ± 1.46	2.0
	FM2	29.63 ± 2.66	9.0
Mg	FM1	879.9 ± 13.6	1.5
	FM2	873.5 ± 20.7	2.4

FM1: fresh beef; FM2: fresh pork; PM1: sliced bovine meat; PM2: sausage; PM3: meatballs.

As can be seen in the Table 4, significant concentrations of Mg were found in the analyzed fresh meat samples. However, lower concentrations were found for samples of processed meat (Table 1). These variations may be due to a number of reasons, which can be related to differences in the nutrient composition of the selected meat products for analysis (based on one particular cut of meat from one breed of cattle), as well as, on the diet of the livestock and on the soil in which the animal feed was grown.[4] Moreover, recent researches have showed losses of nutritional quality of vitamins and minerals during food processing.[8,10,11,34] Changes in mineral contents in meat samples were also observed by Gonçalves *et al*.[35] In this study, the authors verified that thermal processing promoted the reduction of Cu content in analyzed meat samples, besides influencing the chemical form of the element, which might influence its bioavailability.

For Cu, similar concentrations were found between bovine meat samples (processed and fresh, Table 4). However, lower concentrations of this element were found in the two other processed meat samples. This can be related to losses during processing or can be related to the composition of these samples, since both are composed of a mixture of meats, considering that concentrations about three times smaller were found in analyzed pork samples.

The comparative mineral compositions (Ca, Fe and Mg) for all analyzed samples are presented together in the Figure 1. The results of this study showed that the content of these elements widely varies with the type of meat.

Conclusions

The results of the present investigation show that the proposed sample dissolution procedure using TMAH can be applied for the determination of Ca, Fe and Mg by FAAS

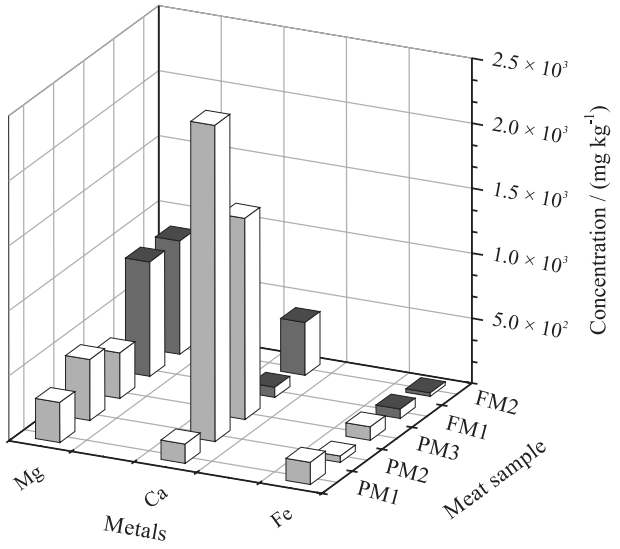

Figure 1. Comparative composition for Ca, Fe and Mg in different samples of meats after alkaline treatment with TMAH, obtained by FAAS (in mg kg^{-1}). FM1: fresh beef; FM2: fresh pork; PM1: sliced bovine meat; PM2: sausage; PM3: meatballs.. For clarity, error bars and significations were omitted in this figure.

and of Cu by HR-CS GFAAS in different meat samples. This procedure compares well with the more conventional nitric acid dissolution for the determination of these elements, showing advantages. The dissolution with TMAH is a fast, simple and reproducible method that does not require especial instrumentation, such as microwave oven, neither strong acid, for sample preparation. Furthermore, this procedure requires small amounts of samples, reagent and is less susceptible to contamination or analyte losses, presenting itself as a methodology for routine analysis.

From these studies, it can be concluded that red meat is a non-homogenous food and that its nutritional composition (Ca, Cu, Fe and Mg) can be dependent on the metal concentration that is naturally present in the meat or on the possible losses and/or gains of elements during industrial processing steps. However, it was possible to verify that red meat is an important source for some micronutrients, being of great nutritional importance for human health and development.

Acknowledgements

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for research scholarship, the Ministério da Agricultura, Pecuária e Abastecimento (CNPq/MAPA/SDA – 578261/2008-1) and to the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS/CNPq – PRONEX – 10/0005-1) for financial support.

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Submitted: March 4, 2011
Published online: July 14, 2011