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Evaluation of Sample Preparation Methods Based on Alkaline and Acid Solubilization for the Determination of Na and K in Meat Samples by Atomic Spectrometric Techniques

Caroline S. da Silva, Adriane M. Nunes, Eliézer Q. Oreste, Tanize S. Acunha, Mariana A. Vieira and Anderson S. Ribeiro*

Universidade Federal de Pelotas, Centro de Ciências Químicas, Farmacêuticas e Alimentos, Programa de Pós-Graduação em Química, Laboratório de Metrologia Química, 96160-000 Capão do Leão-RS, Brazil

Foram avaliados dois métodos diferentes de preparo de amostras de carnes baseados na solubilização em meios alcalino ou ácido. O objetivo deste estudo foi determinar os teores de Na e K após solubilização com hidróxido de tetrametilamônio (TMAH) à temperatura ambiente, e com ácido fórmico e aquecimento em bloco de digestão a 50 ºC por cerca de 2 h. A solubilização alcalina se apresentou como uma metodologia simples e robusta e a melhor alternativa frente aos procedimentos de mineralização convencional, permitindo a solubilização completa das amostras usando pequenas quantidades de TMAH. O método foi validado empregando-se materiais de referência certificada assim como pela comparação com método de digestão convencional com ácido nítrico. Os limites de detecção obtidos foram de 0,8 e 2,0 mg g^{-1} para Na e K, respectivamente, e se mostraram adequados para o objetivo das análises.

Two different sample preparation methods were evaluated for meat samples employing the solubilization in alkaline or acidic media. The aim of this study was to determine Na and K levels after sample solubilization with tetramethylammonium hydroxide (TMAH) at room temperature, and with formic acid and heating in a digester block at 50 ºC for ca. 2 h. The alkaline solubilization showed to be a simple and robust method and the better alternative in relation to the mineralization conventional procedures, allowing the complete solubilization of the samples even when small quantities of TMAH were used. The method was validated by using certified reference material as well as by comparing with samples digested with nitric acid. The limits of detection obtained were 0.8 and 2.0 μg g^{-1} for Na and K, respectively, and showed to be adequate for the analysis purposes.

Keywords: processed meat, sodium, potassium, alkaline and acid solubilization, atomic spectrometry

Introduction

Sodium chloride is one of the most common ingredients used for meat preservation. Its concentration has an important role in the microbial growth control and usually improves the meat sensorial perception. In general, the saltiness perception is very well accepted by consumers and it can improve the flavor of other components, naturally present in the food.[1,2] However, it is known that Na ions present in food may increase the risk of hypertension. Thus, a lower salt consumption has been recommended and the level of Na needs to be evaluated in food, especially in common products.[3] In addition, clinical and epidemiologic studies suggest that blood K levels also contribute to the blood pressure regulation.[3,4] On the other hand, food industries are replacing the maximum allowed Na levels by K salts[5-9] as well as other substances such as synthetic antioxidants (ascorbic acid, nitrite and phosphate), although the use of natural antioxidants has already been evaluated and can be used for meat products (culinary herbs/spices, fruits, vegetables and oil seed products, among others). Results of related studies have demonstrated that the reduction of NaCl leads to a reduction in both flavor acceptance and meat preservation, requiring further investigations.[10-14]

*e-mail: andersonsr@pq.cnpq.br

The most conventional analytical methods used to prepare biological tissue samples prior to the determination of metals predominantly involve the digestion of the sample with oxidant acids and heating (in digester blocks or assisted by microwave radiation).[15-17] However, most of these procedures are tedious, require complex laboratory equipment, and increase the risk of contamination and analyte loss by volatilization or adsorption in glass flasks. In this context, simple alternatives to avoid these potential problems include the direct analysis of 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.[18,19] However, this kind of procedure is not used for routine analysis and is still a relatively new approach in areas in which the analyses are not carried out by analytical chemists. Regarding the slurry sampling, the methods involved are particularly attractive because they combine advantages of the direct sampling (ease to prepare, no requirement of aggressive chemical treatments, less susceptibility to contamination and reduction of analyte losses before analysis) with the liquid sampling facilities, allowing the use of aqueous standards for calibration curves. However, their stabilization, homogeneity, particle size and sedimentation are parameters that must be taken into consideration. Thus, these methods involve the complete or partial solubilization of the sample matrix and both acids and alkaline reagents can be used.[20-24]

Recently, tetramethylammonium hydroxide (TMAH) has been used to solubilize biological samples.[18,21,22] Biological samples treated with TMAH provide suspensions that remain stable from days to months at room temperature.[23,24] This treatment results in a very simple method that generally does not require any heating and therefore prevents the loss of volatile analytes before analysis. Furthermore, only small amounts of TMAH solution are necessary for complete solubilization of the samples, resulting in smaller dilution volumes, which is very important in the case of trace analysis.[21]

In relation to the acidic reagents, formic acid (HCOOH) has been widely used as an alternative reagent since it is easy to obtain and safe for sample preparation, especially for the solubilization of biological tissues before metal determination. In addition, it can be used in closed flasks and in most cases without the need of external energy for solubilization of the samples, while the assistance of ultrasound can be used to reduce acid consumption and sample preparation time.[25-29]

Considering the importance of certain analytical data to both consumers and health professionals, and also the recent legislations for Na concentration in foods, this work focus on the sample preparation of meat based products in order to simplify the procedures available for the determination of Na and K by atomic spectrometric techniques. For this purpose, two methods were evaluated to prepare a slurry sample: (*i*) using the alkaline solubilization with TMAH and (*ii*) using acidic solubilization with formic acid in order to determine the better alternative method in relation to the conventional procedures of mineralization. The method proposed was validated by the determination of metals in certified reference material as well as by the analysis of samples prepared using a comparative method, which was the conventional digestion with oxidizing acid.

Experimental

Instrumental

The measurements for Na and K were carried out using different spectrometers, as described below.

The spectrometer 1 (FAES 1 or FAAS (flame atomic emission and flame atomic absorption spectrometer, respectively)) was a Model AA-6300 atomic absorption with flame (Shimadzu, Japan) and with Smith-Hieftje background correction, operating in both atomic emission or absorption modes, and it was used for the determination of Na and K in commercial samples of processed meat. An air-acetylene flame was used for all determinations. The spectrometer was operated using wavelengths of 589.0 and 766.5 nm and using a spectral band path of 0.2 and 0.5 nm for Na and K determinations, respectively. The lamp current was 8 mA/600 mA for both elements. The spectrometer 2 (FAES 2) was a flame emission photometer Model B462 (Micronal, São Paulo-SP, Brazil), operating at the following conditions: sample volume (5 mL min^{-1}), settling time of reading (8 s), air (9 L min^{-1}) at a pressure of 1 kgf cm^{-2} and butane gas flame (liquefied petroleum gas).

All 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 acid digestion sample, a heated digester block was used (MA-4025 Marconi, Piracicaba-SP, Brazil).

Reagents and samples

Analytical reagent grade materials were used for all of the experiments. Ultrapure water was used to prepare all the solutions and it was obtained employing a Direct-Q 3 Water Purification System (Millipore Corporation, Bedford, MA, USA), with a resistivity of 18.3 MΩ cm. Distilled nitric acid (Synth, Brazil) was used and for its purification a MA-075 sub-boiling quartz system (Marconi, Piracicaba-SP, Brazil) was employed. Before using, all glass apparatus were conventionally washed and soaked in 10% (v/v) HNO_3

for at least 48 h, and then rinsed with ultrapure water prior to use. Working reference solutions of Na and K were daily prepared by appropriate dilutions of a stock solution containing 1000 mg L^{-1} in ultrapure water from a standard concentrate solution (Fluka Analytical, Germany). For sample digestion, the following reagents were used: formic acid (Fluka Analytical, Germany), tetramethylammonium hydroxide pentahydrate (Sigma Aldrich, Germany), 35% (v/v) hydrogen peroxide (Fluka Analytical, Germany) and nitric acid (Synth, Brazil). For the analysis using the spectrometer 1 operating at absorption mode, a buffer solution of CsCl 0.09% (m/v) was added in samples and standard solutions to minimize the ionization interference.

For both method development and analyte monitoration, different meat samples from Brazilian manufacturers were used. These samples were initially cut and homogenized using a blender (non-contaminating kitchen mixer). They were analyzed in triplicate, immediately after using the sample preparation methods, or put in cleaned plastic pots and frozen at −16 °C and naturally defrosted just before analysis. The certified reference material (CRM) SRM 1546 Meat Homogenate produced by the National Institute of Standards and Technology (NIST) was used in this work in order to validate the method.

Sample preparation procedures

Samples were prepared using three different procedures as described below. Procedure 3 was used in order to verify the accuracy of the results since it is the reference method for meat digestion according to the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) (Normative Instruction No. 400/03) for the determination of trace metals in muscle, liver and kidney.

Solubilization with TMAH (Procedure 1)

Samples were prepared in polyethylene flasks by mixing approximately 250 mg of the processed meat sample with 400 µL of a 25% (m/v) TMAH solution in water. The slurry was left standing overnight at room temperature and remained closed until achieving the complete solubilization. The volume was filled up to 50 mL with deionized water, and thus the final TMAH concentration was 0.2% (m/v). The obtained sample mixture was yellow to brown in color, resembling the original color of the sample, as turbid slurry.

Solubilization with Formic Acid (Procedure 2)

Processed meat samples were treated with formic acid for their solubilization based on the procedure proposed by Scriver *et al.*[26] The same sample mass used in the previous procedure was weighed into glass tubes, then 10 mL of concentrated formic acid were added, and the mixture was heated in a digester block at 50 ºC for ca. 2 h. After cooling, this solution was transferred to a 50 mL volumetric flask, and the volume was completed with deionized water for subsequent analysis.

Acid digestion with HNO_3/H_2O_2 (Procedure 3)

Processed meat samples were treated with an acid digestion. The decomposition was carried out in open tubes in an aluminum heating block. For this procedure, approximately 250 mg samples were weighed into glass digester flasks, then 2.5 mL of concentrated nitric acid were added, and the mixture was heated in a digester block at 90 ºC for 1 h. After cooling at room temperature, hydrogen peroxide (2.0 mL) was added, and the mixture was heated at the same temperature for an additional 1 h. The digestion was complete when all the meat fat had visually dissolved. After cooling, the flask was filled up to a volume of 50 mL with deionized water for subsequent analysis.

Results and Discussion

Comparison of the solubilization methods proposed

To optimize the operating conditions for the determination of Na and K by FAES using the solubilization with TMAH and formic acid, different masses of samples were investigated to verify problems related to the homogeneity. For this study, masses ranging from 0.25 to 0.75 g were evaluated for a test sample. In this study, it was observed that even for different sample masses, the solubilization with TMAH or formic acid allowed to obtain homogeneous sample solutions, which presented relative standard deviations (RSD) < 5.0%. Thus, based on these data, a minimum mass of 250 mg was established.

Analytical results were obtained by preparing the calibration curves in the same medium used for the sample decomposition (TMAH, formic acid or diluted nitric acid). Samples were diluted with deionized water to be within the linear calibration range. The figures of merit obtained by FAES for the calibration curves for Na and K are shown in Table 1. Good linearity for such curves was obtained for both analytes ($r > 0.999$) independent of the method used for sample preparation. The sensitivities, given by the slope of the curves, were close for the different media studied. Also, the limits of detection (LOD) in the measuring solutions (defined as the concentration equivalent to three times the standard deviation of 10 measurements of the blank on the sensitivity curve) were of the same order of magnitude, and there was no significant difference between the various sample preparation methods. LOD for the

Table 1. Figures of merit for the determination of Na and K in processed meat samples by flame atomic emission spectrometry (FAES 1) after treatment with TMAH, formic acid or nitric acid

Analyte	Range / (mg L^{-1})	TMAH			Formic acid			HNO_3		
		a	LOD / (μg L^{-1})		a	LOD / (μg L^{-1})		a	LOD / (μg L^{-1})	
K	0.2-0.8	0.5922	4.6[a]	2.0[b]	0.5697	5.7[a]	3.0[b]	0.6048	2.8[a]	1.5[b]
Na	0.1-0.6	0.8679	1.4[a]	0.8[b]	0.8003	3.6[a]	2.0[b]	0.8275	1.0[a]	0.6[b]

a: slope of the calibration curve (L mg^{-1}); LOD: limit of detection; [a]instrumental; [b]original sample.

original sample was calculated considering the mass and the sample dilution factor used.

In order to obtain information about the accuracy of the results, a comparison between the proposed methods (Procedures 1 and 2) and the reference method (Procedure 3) was performed. Samples were analyzed by FAES or FAAS. The obtained results are presented in Table 2.

Concentrations obtained from the analysis of commercial meat samples (sliced bovine meat, Vienna sausage and meatballs*)* using the three procedures of sample preparation were submitted to a statistical paired *t*-test. This is an analogy to the normal *t*-test, in which the data are analyzed in pairs (with a 95% confidence level). Results obtained in the medium of formic acid compared to the conventional nitric acid dissolution indicated that the two methods provide different results for K, and the level of probability was 99.0 and 99.9% for sausage and meatballs, respectively. However, the concentration measured for both elements in the medium of TMAH compared with the digestion method showed no significant differences for 95% confidence level. This means that this latter procedure is more appropriate for the preparation of meat samples. Thus, the methodology based on the solubilization of the meat samples in alkaline medium for determination of Na and K was subjected to different validation procedures.

In order to evaluate the accuracy of the results from the proposed method with TMAH, the analyses of these samples were made for both analytes using FAAS and another flame photometer instrument (FAES 2). According to these results, it was verified that the values of the concentration of both elements, independent on the analytical technique used, were in agreement with those previously obtained, which proves the veracity of the results. The statistical comparison by paired *t*-test (95% confidence level) showed no significant difference between these results (Table 2).

Similar results were obtained for the different types of meat treated with TMAH and formic acid. In the presence of these reagents, the resulting opaque solutions indicated a slurry formation. However, the comparison of the two approaches revealed peculiarities for each treatment: the solubilization with formic acid required a mild heating to the complete dissolution of the samples. Alternatively, the use of TMAH led to a sample solubilization through a reaction proceeded at room temperature (overnight), thus eliminating the use of any heating. Regarding the amount of reagents, 10 mL of concentrated formic acid were necessary, while lower quantities of TMAH were used. In this case, the reagent volume used was exactly 20 times lower. The use of small amounts of TMAH is important for preventing problems of health of the analyst, even though TMAH has a low toxicity and some precautions are needed such as use of a good exhaust system.[30] Some sample measurements were performed in TMAH and it was observed that the signal for analytes remained stable for at least 3 months, which corroborates the data reported in literature.[21,23,24] On the other hand, there is an advantage on the solubilization with formic acid that eliminates a minimal odor during sample preparation. Both approaches provide

Table 2. Analytical results (mean value ± SD (mg g^{-1}) (RSD, %)) for processed meat samples after treatment with different sample preparation methods and determination using different instruments

Analyte	Sample	TMAH			Formic acid	HNO_3
		FAES 1	FAES 2	FAAS	FAES 1	
K	A	1.09 ± 0.06 (5.5)	1.19 ± 0.10 (8.4)	1.10 ± 0.06 (5.5)	1.15 ± 0.01 (0.9)	1.06 ± 0.02 (1.9)
	B	0.92 ± 0.02 (2.2)	0.97 ± 0.01 (1.0)	0.89 ± 0.01 (1.1)	1.01 ± 0.02 (2.0)	0.90 ± 0.01 (1.1)
	C	1.58 ± 0.06 (3.8)	1.56 ± 0.14 (9.0)	1.53 ± 0.05 (3.3)	1.65 ± 0.01 (0.6)	1.51 ± 0.01 (0.7)
Na	A	4.65 ± 0.02 (0.4)	4.63 ± 0.18 (3.9)	4.53 ± 0.06 (1.3)	4.82 ± 0.06 (1.2)	4.71 ± 0.02 (0.4)
	B	8.57 ± 0.03 (0.4)	8.94 ± 0.16 (1.8)	8.29 ± 0.14 (1.7)	8.83 ± 0.08 (0.9)	8.66 ± 0.05 (0.6)
	C	3.05 ± 0.02 (0.7)	2.85 ± 0.05 (1.7)	3.11 ± 0.02 (0.6)	2.89 ± 0.03 (1.0)	2.81 ± 0.05 (1.8)

A: sliced bovine meat; B: vienna sausage; C: meatballs; SD: standard deviation; RSD: relative standard deviation.

simple, fast and inexpensive alternatives when compared with the traditional techniques used for food analysis. Furthermore, slurries obtained can be directly introduced into different equipments used for Na and K determination.

Recovery tests in commercial samples were carried out by adding analytical solutions of Na and K to the samples (equivalent to 0.20 mg L^{-1} for both analytes) before the solubilization with TMAH. These analytes were determined by the method proposed and the recoveries obtained varied from 90 to 100%. The concentrations obtained for the certified reference material (SRM 1546 - meat homogenate) are presented in Table 3. Results showed good agreement with the certified values for both analytes, presenting RSD < 5.0%, which demonstrates the potentiality of using TMAH for sample preparation of meat. The statistical comparison by *t*-test (95% confidence level) showed no significant difference between these results.

Table 3. Analytical results (mg kg^{-1}) for Na and K in certified reference material (SRM 1546, meat homogenate) after sample treatment with TMAH

Analyte	Found / (mg kg^{-1})	RSD / %	Certified / (mg kg^{-1})
K	2219 ± 5	0.22	2370 ± 200
Na	10011 ± 205	2.05	9990 ± 716

RSD: relative standard deviation.

Analysis of the different samples of meat by alkaline solubilization

The availability of this method was demonstrated by the analysis of 19 samples from different categories of processed meat. This study showed that the proposed method was not only adequate but also robust since all the samples could be easily solubilized in such conditions. As it can be observed in Table 4, good precisions were obtained, with RSD < 4% for all samples, independent of the analyte. The results were submitted to statistical test (*t*-test, 95% confidence level) and no significant difference between results.

As mentioned in the literature, salt is one of the main ingredients added to industrialized food. In meat products, the functions of the salt (added in a 2.5 to 3.0%) are to inhibit microorganism growth, to enhance the flavor and also to extract salt soluble proteins.[1,2] According to the results (Table 4), it can be seen that all the processed meat

Table 4. Analytical results (mean value ± SD (mg g^{-1}) (RSD, %)) for Na and K in different categories of meat after sample treatment with TMAH and determination by FAES 1

Sample	Na			K
	Found	Label	Targets for 2012[a]	Found
Beef burger	6.20 ± 0.01 (0.16)	8.42	3.00	3.72 ± 0.04 (1.08)
Bologna	8.92 ± 0.06 (0.67)	13.75	6.00	1.46 ± 0.04 (2.74)
Ham 1	5.47 ± 0.08 (1.46)	8.32	6.50	3.53 ± 0.03 (0.85)
Ham 2	7.44 ± 0.05 (0.67)	2.02	6.50	2.36 ± 0.04 (1.70)
Ham 3	7.78 ± 0.16 (2.06)	9.82	6.50	2.36 ± 0.01 (0.42)
Kibbeh	4.74 ± 0.11 (2.32)	6.90	3.00	2.72 ± 0.04 (1.47)
Meetballs	2.85 ± 0.05 (1.75)	0.68	3.00	1.66 ± 0.01 (0.60)
Pepperoni sausage	10.33 ± 0.17 (1.65)	10.60	6.00	3.04 ± 0.01 (0.33)
Pork burger	5.92 ± 0.09 (1.52)	10.57	3.00	3.20 ± 0.01 (0.31)
Sausages bock	9.62 ± 0.18 (1.87)	10.74	6.00	2.30 ± 0.03 (1.30)
Sausages frankfurters	6.00 ± 0.28 (4.67)	5.50	5.50	1.49 ± 0.03 (2.01)
Sausages hot dog	10.73 ± 0.38 (3.54)	15.72	6.00	3.60 ± 0.06 (1.67)
Sausages peritif	4.61 ± 0.01 (0.15)	11.76	5.50	0.96 ± 0.01 (1.04)
Sausages Vienna	8.94 ± 0.16 (1.79)	11.70	5.50	1.00 ± 0.01 (1.00)
Sliced bovine meat	4.63 ± 0.18 (3.89)	5.90	4.50	1.33 ± 0.01 (0.75)
Smoked bologna	11.24 ± 0.34 (3.02)	7.80	6.00	2.42 ± 0.03 (1.24)
Smoked sausage	11.29 ± 0.06 (0.53)	14.94	6.00	3.10 ± 0.04 (1.29)
Stomach bovine	2.45 ± 0.09 (3.67)	0.75	4.00	1.08 ± 0.01 (0.93)
Traditional bologna	10.54 ± 0.16 (1.52)	11.68	6.00	2.45 ± 0.04 (1.63)

[a]Value established (mg g^{-1}) by Food Standards Agency (UK);[31] SD: standard deviation; RSD: relative standard deviation.

samples showed high concentrations of Na and a relatively low concentration of K. The low concentration of K can increase the risk of developing hypertension, considering that a diet low in K and high in Na may lead to high blood pressure and so an "equal" amount of these elements should be targeted.[7]

Among the analyzed samples, bologna, ham and some categories of sausage showed the highest concentrations of Na, which can be attributed to the use of high proportions of additives such as sodium nitrite/nitrate (preservative), sodium phosphate (stabilizer) and sodium erythorbate (antioxidant).[3] Furthermore, according to these results, some samples presented Na concentrations two times higher than the current targets for 2012, considering the Food Standards Agency (FSA)[31] from United Kingdom recommendations.

Regarding the levels of Na, it is also necessary to mention the difference between the information of the concentrations provided on the packaging and the values obtained in the analysis. Some meat samples showed high Na concentrations (over 100%) regarding the established values. The Brazilian Health Surveillance Agency (ANVISA) set a tolerance value up to 20% in relation to the ones declared on the product labels.[32] For products containing an excess of micronutrients (values in excess of the limit), the company should keep the disposition studies to justify such variation.[32]

Results reported for Na also showed that only four samples are in agreement with the Brazilian specification. For all samples, K concentrations were, on average, 34% above the concentrations found for Na, showing that the Brazilian industries are not reducing the level of Na yet, what could be done by substituting sodium salts for potassium salts.

Finally, the results above mentioned highlight the necessity of a more effective quality control from responsible organization, as well as the applicability of robust analytical methodologies, which can also be sensitive and reliable by coupling an adequate sample preparation with optimized instrument conditions.

Conclusions

One of the focuses of this study was to analyze food products that may contribute significantly to the total salt intake. The high concentrations of Na found in the analyzed meat samples and the differences between these levels and the ones reported on the labels emphasize the importance of having adequate methods for routine analysis as well as the salt content reducing in processed foods in Brazil.

Compared to the conventional sample preparation method used to determine metals in meat products, TMAH showed to be very simple, reproducible and promoting the complete solubilization of the different samples studied. This method is less susceptible to contamination or analyte losses by volatile species or loss in the surface of the flasks, uses small amounts of reagent and sample, and presents low risks to the health of the analyzer. It is also very adequate to be used in routine analysis and contributes to the green analytical chemistry.

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