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SHORT COMMUNICATION

Capillary electrophoresis to approach sorbate usage in processed meat products in Brazil

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Abstract Sorbate is a highly used preservative in a wide range of processed foods, including meat products. In this work sorbate usage in commercial processed meat products from the Brazilian market was evaluated. A capillary electrophoresis method for sorbate analysis using ethanol:water extraction solution (1:2, v/v) and sodium tetraborate (20 mmol L^{-1}) electrolyte solution was validated. Low limits of detection (0.4 mg L^{-1}) and quantification (1.0 mg L^{-1}), good precision (RSD = 3.6%) and suitable accuracy (70.2%, RSD = 1.8%) were attained. Linearity was observed from 1.0 to 15.8 mg L^{-1}, with $r \geq 0.999$. The proposed method was applied to Brazilian pork and hot dog sausages, salami, ham and mortadella. A peak of sorbate between 4 and 6 min was found in pork and hot dog sausages, poultry and pork mortadella, but not in ham and salami. The sorbate levels ranged from 54.0 to 976.4 mg kg^{-1}. Sorbate concentration exceeded the 200 mg kg^{-1} limit of the Brazilian legislation in all sorts of products in the majority of the brands despite there being no information regarding sorbate on the label. These results indicated the widespread use of sorbate, turning this into food for thought.

Keywords Capillary electrophoresis · Sorbate · Processed meat products · Preservatives

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Introduction

Processed meat products are produced mainly from pork and poultry. These products are highly consumed worldwide as well as by the Brazilian population, especially ham, mortadella and sausages (Datamark 2010). About 3.5 million ton of pork meat were produced in 2014 in Brazil, of which 86% were directed to the production of processed meat foodstuffs (IBGE 2015). The annual per capita consumption of pork meat in 2015 was 11.7 kg in Brazil, 21.7 kg in the US and 31.0 kg in the European Union (OECD 2015).

Additives such as nitrite and nitrate are indispensable for processed meat products to provide the typical colour and flavour of cured meat and to avoid deterioration; they are particularly effective against *Clostridium botulinum* (Yu et al. 2015; Keeton 2011). Sorbic acid and sorbate are largely used as preservatives in a wide range of foods including meat, dairy and fruit products, bakery, sauces, beverages and sweeteners. The use of sorbate in meat products is usually associated with nitrite (Jaraith et al. 2015; Mahboubifar et al. 2011). The widespread use of sorbate in foods has become food for thought. It should be noted that after reassessment, the acceptable daily intake (ADI) of sorbate was reduced from 25 (JECFA 1974) to 3 mg per kg body weight daily (EFSA 2015).

The presence of both sorbate and nitrite salts in meat products might result in the formation of mutagenic and genotoxic species (EFSA 2015; Osawa et al. 1986), increasing the potential risk of cured meats consumption to human health. Processed meat was recognized as carcinogenic based on sufficient evidence that its consumption causes colorectal cancer in humans. Data provided by several studies led to the conclusion that 34,000 deaths caused by cancer worldwide can be associated to the intake

of processed meat. If consumed daily, a portion of 50 g of processed meat might increase in 18% the risk of colorectal cancer development (IARC 2015a, b).

In this scenario, analytical methods that provide reliable and accurate data on preservatives in foods are essential and several have been reported. UV/Vis spectrophotometry and liquid chromatography techniques are the most used to determine sorbate in food matrices, such as dairy products (Amirpour et al. 2015; Javanmardi et al. 2015; Ulca et al. 2013; Tfouni and Toledo 2002; AOAC 1990), wine (Ulca et al. 2013; Machado et al. 2007; Mihyar et al. 1999; AOAC 1990), other beverages (Amirpour et al. 2015; Ulca et al. 2013; Tfouni and Toledo 2002; AOAC 1990), and salami (Holley and Millard 1980). Both techniques are time-consuming, require the use of organic solvents and sample pretreatments.

Capillary electrophoresis is a separation technique based on differences in the mobility of ionized species submitted to an electric field (Skoog et al. 2006). Capillary zone electrophoresis enables separation of inorganic ions and ionized organic molecules applying suitable water based electrolytes and avoiding the use of organic solvents. Besides high resolution and ease of operation, it also provides reduced time of analysis and sample preparation (Skoog et al. 2006; Gatea et al. 2015). Capillary electrophoresis has been reported to analyze sorbic and benzoic acids and their salts in soy sauces, vinegar and soft drinks and juices (Petruci et al. 2011).

The aim of this work was to identify and quantify sorbate, and to evaluate its usage in commercial processed meat products from the Brazilian market.

Materials and methods

Chemicals

Sorbic acid and sodium tetraborate were obtained from ProSynth (Acton, Suffolk, UK) and Synth (Diadema, São Paulo, Brazil), respectively. Water was purified using a Milli-Q purification system (Millipore, Bedford, MA, USA). Ethanol was obtained from Qhemis (Indaiatuba, SP, Brazil).

Samples

Pork and hot dog sausages, mortadella (poultry and pork), salami (traditional and pepperoni) and ham (traditional and a typical Brazilian ham-like product) samples manufactured by the most representative brands of the Brazilian market were obtained from local supermarkets in Araraquara, SP, Brazil. Three brands of ham, ham-like and pork sausages, two brands of traditional and one of pepperoni salami, two brands of hot dog sausage and one brand of mortadella (pork and poultry) were selected. Up to three packages of each brand with different expiration dates were purchased.

Sample preparation

First, each sample was ground and homogenized in a domestic food processor (Walita Paris, Phillips, Brazil). Samples were weighted (0.5000 g) and placed in a tube with ethanol:water extraction solution (1:2, v/v), homogenized in vortex (1 min) and put in an ultrasonic bath (20 min). After filtration (Whatman n° 1), the extract was collected and volume was adjusted (25.00 mL). Extracts were then filtered through Nylon disk filters (0.45 μm) and injected into the capillary electrophoresis system. Each sample was extracted twice and each extract was injected twice ($n = 4$). Samples were extracted, suitably diluted and injected in the same day.

Capillary electrophoresis analysis

Experiments were performed using a capillary electrophoresis system (HP 3D, Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector and a temperature control device. Data acquisition and instrumental conditions were controlled using the HP ChemStation® software. Fused-silica capillaries (48.5 cm total length, 40 cm effective length, 75 μm i.d. × 375 μm o.d.) were obtained from Polymicro Technologies (Phoenix, AZ, USA). Sample extracts were injected hydrodynamically at 30 mbar for 8 s, 20 kV current was used and detection wavelength was set at 255 nm. The temperature was 25 °C and total run time was 7 min. The electrolyte was a solution of sodium tetraborate (20 mmol L^{-1}) at pH of 9.3. The capillary was conditioned daily by flushing a sodium hydroxide solution (1 mol L^{-1}) for 5 min, followed by purified water for 5 min and then electrolyte solution for 15 min. In between runs the capillary was flushed with electrolyte solution for 1 min.

Electropherograms of the sample extracts were compared to those of a standard solution of sorbic acid. The UV/Vis spectra were also compared to confirm the identity. For quantification purposes calibration curves (peak area versus sorbate concentration) were prepared daily, in triplicate, using standard solutions in five concentrations.

Validation

After establishing extraction and capillary electrophoresis conditions, validation was carried out according to protocols reported in literature (Eurachem 2014; IUPAC 2002). Calibration, linearity, limit of detection, limit of quantification, precision and accuracy were evaluated.

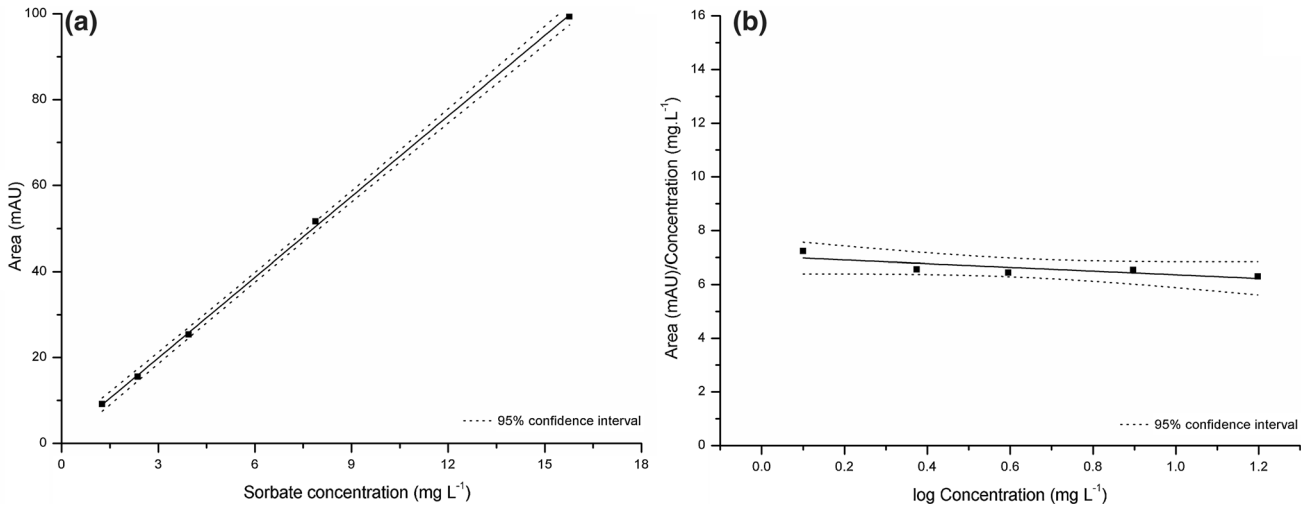

Fig. 1 Typical area versus sorbate concentration calibration curve (1.3–15.8 mg L^{-1}), y = 6.25251x + 1.14907, r = 0.99982 (**a**) and area/concentration ratio versus log of sorbate concentration (**b**) with 95% CI

Data analysis

Results were expressed as mean ± standard deviation. Origin 8.0 Software (OriginLab Corp, MA, USA) was used to obtain linear regression and correlation coefficients. All data obtained was submitted to ANOVA and Tukey test or *t* test using Microsoft Excel 2013 (Microsoft Corporation, WA, USA) and BioEstat 5.3 (Instituto Mamirauá, AM, Brazil).

Results and discussion

Experimental conditions

Capillary electrophoresis separation is due to the difference in ion mobility under specific electric field conditions. Organic molecules with protonated groups can be ionized by controlling the pH of an aqueous solution. Sorbate has a carboxylic group with pKa 4.8 and ionization can be satisfactorily performed above pH 5.0. Sodium tetraborate solution at 20 mmol L^{-1} shows a pH value of 9.3 and was therefore the electrolyte selected, based on the work of Petruci et al. (2011). Capillary zone electrophoresis analysis parameters according to Petruci et al. (2011) were tested and adjustments were needed in order to attain good resolution in the electropherograms. The initial temperature of 29 °C was lowered to 25 °C and run time was extended to 7 min. Wavelength detection was set to 255 nm, the maximum absorption for sorbate. An electric current of 20 kV and hydrodynamic injection of 30 mbar for 8 s were used.

For sorbate extraction, ethanol and different proportions of a solution of ethanol:water (1:1 and 1:2, v/v) were tested. The ethanol:water solution of 1:2 (v/v) showed better resolution and intensity of the sorbate peak, as well as better maintenance of the electric current during run time. Ethanol and the ethanol:water solution of 1:1 (v/v) could not sustain electric current. The presence of large amounts of ethanol in the capillary makes it difficult for the electric current to be maintained, but ethanol is needed to assure that all of the sorbate is extracted from fatty food matrices such as processed meat products. The ethanol:water solution of 1:2 (v/v) was selected because it represented a compromise considering the analytical signal intensity and current counts.

Validation

Laboratory validation was performed by evaluating analytical figure of merits. Precision was calculated using the relative peak area repeatability for six injections of a standard solution of sorbate (1.0 mg L^{-1}). The overall repeatability showed a RSD of 3.6%. Linearity was observed in the sorbate concentration interval of 1.0–15.8 mg L^{-1} (equivalent to 50.0–788.5 mg sorbate kg^{-1} meat product). Linear regression equations (y = a + bx) and linear correlation coefficients, r ≥ 0.999, were obtained (Fig. 1a). Linearity was also studied using the area/concentration ratio versus sorbate concentration in the calibration curve, expressed in logarithmic scale. All concentrations used in the calibration curve were within 95% CI (Fig. 1b) (Nasser et al. 2005). Limit of detection was calculated as 0.4 mg L^{-1} (equivalent to 19.5 mg kg^{-1}). Limit of quantification was 1.0 mg L^{-1} (equivalent to 50.0 mg kg^{-1}). Recovery was performed to evaluate the accuracy of the method using two spiking levels (1.8 and 8.0 mg L^{-1}; equivalent to 90 and 400 mg kg^{-1}). Recovery was expressed as the percentage of the sorbate

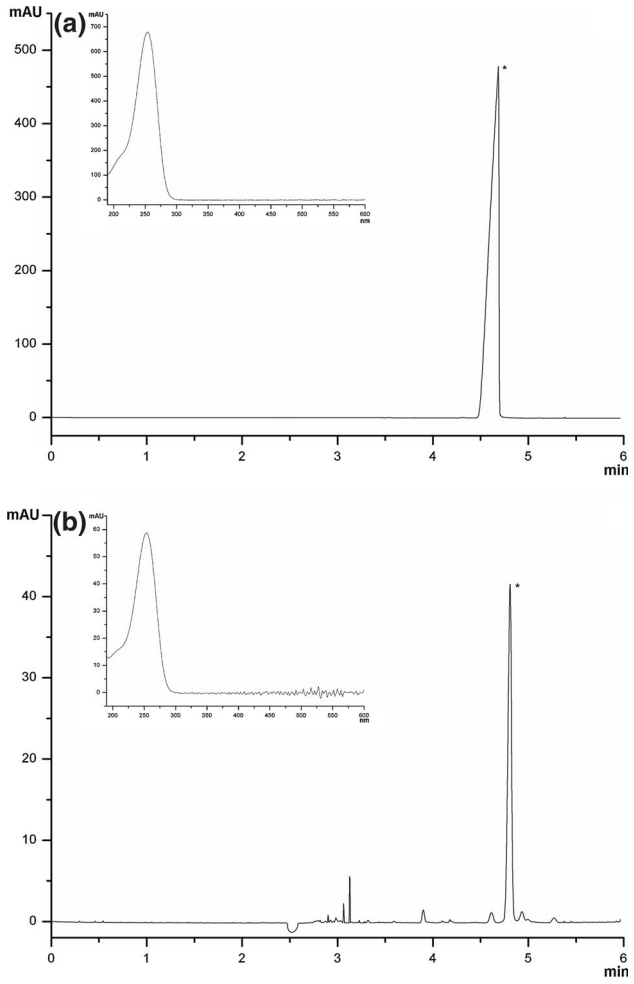

Fig. 2 Typical electropherograms and sorbate UV/Vis spectra of sorbate standard solution (1000 mg L^{-1}) (**a**) and poultry mortadella extract (**b**). Sorbate peak (*). Electrophoretic conditions according to experimental conditions section

concentration obtained relative to the amount of sorbate added to the sample. The minimum recovery was 70.2%, with maximum RSD of 1.8%, showing that even at a low concentration level suitable accuracy was attained.

Analysis of Brazilian meat products

The method was employed to evaluate sorbate in Brazilian processed meat products. Figure 2 shows typical electropherograms of a sorbate standard solution and of a poultry mortadella extract, with the correspondent UV/Vis spectra of the sorbate peaks.

A sorbate peak between 4 and 6 min was obtained in two of the three pork sausage brands, both hot dog sausage brands, and pork and poultry mortadella, with a correspondent UV/Vis spectrum with the same profile as the sorbic acid standard. Two brands of ham and all salami brands did not show a peak for sorbate, indicating therefore that they did not contain sorbate, or sorbate levels were below the limit of detection. All sample extracts were spiked with a standard solution of sorbate to confirm peak retention time. One of the ham brands showed bad resolution in the electropherogram, even after adjustments. It occurred probably because of the excessive amount of fat in the extract, which prevented electrolyte flow in the capillary leading to a decay in electric current. Sorbate levels in this sample could not be analyzed.

The sorbate levels of pork and hot dog sausages and mortadella are in Table 1. Pork and hot dog sausages showed a great range of sorbate levels varying from 54.0 to 440.4 mg kg^{-1}. Sorbate levels in poultry and pork mortadella were even higher, ranging from 378.7 to 976.4 mg kg^{-1}. There was difference in sorbate levels within packages of all the brands ($p \leq 0.05$). Significant differences were also found among brands of pork and hot dog sausage ($p \leq 0.05$). No difference in sorbate levels was found between poultry and pork mortadella ($p > 0.05$).

The sorbate levels of at least one package of all brands of processed meat products exceeded the limit of 200 mg kg^{-1} established by the Brazilian Health Surveillance Agency, with the exception of brand 1 of hot dog sausages. It is also worrisome that these meat products usually contain nitrite and/or nitrate in their composition. It

Table 1 Levels of sorbate (mg kg^{-1}) of Brazilian meat products

Package	Pork sausage		Hot dog sausage		Pork mortadella	Poultry mortadella
	Brand 1	Brand 2	Brand 1	Brand 2	Brand 1	Brand 1
1	$268.5^{aB} \pm 6.5$	$393.4^{bA} \pm 1.1$	$113.2^{aB} \pm 4.5$	$328.0^{aA} \pm 2.4$	$976.4^{aA} \pm 22.4$	$945.5^{aA} \pm 14.6$
2	$60.9^{bB} \pm 2.6$	$440.4^{aA} \pm 1.3$	$76.9^{bB} \pm 1.8$	$257.5^{bA} \pm 1.1$	$548.8^{bA} \pm 1.3$	$616.0^{bA} \pm 17.4$
3	$54.0^{bB} \pm 0.9$	–	–	–	$378.7^{cA} \pm 0.8$	$531.9^{cA} \pm 26.7$

Mean ± SD (n = 4)

Not analysed

Same lowercase letters in the same column did not show difference on Tukey test ($p \leq 0.05$) or *t* student test ($p \leq 0.05$)

Same capital letters among brands of each product did not show difference on *t* student test ($p \leq 0.05$)

should also be noticed that neither of the evaluated products listed sorbate as an ingredient on the label.

A more widespread research should be performed to verify sorbate usage in a wider range of Brazilian processed meat products. Up to now, there are no published studies regarding sorbate in Brazilian meat products.

Conclusion

A capillary electrophoresis method for sorbate analysis in processed meat products can be performed within 7 min using an extraction solution of ethanol:water. Low limits of detection and quantification, good precision and accuracy were attained. Sorbate was found in pork and hot dog sausages, poultry and pork mortadella, but not in ham and salami. Sorbate concentration exceeded the Brazilian legislation limit in all sorts of products in the majority of the brands. No information regarding sorbate usage was found on the label. These results call attention to the widespread use of sorbate, turning this into food for thought.

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References

Amirpour M, Arman A, Yolmeh A, Azam MA, Moradi-Khatoonabadi Z (2015) Sodium benzoate and potassium sorbate preservatives in food stuffs in Iran. Food Addit Contam 8:142–148

AOAC Association of Official Analytical Chemists (1990) Official methods of analysis. AOAC, Virginia

Datamark Market Intelligence Brazil (2010) Carne Industrializada: Carne industrializada. http://www.datamark.com.br/. Acessed 9 Aug 2010

EFSA European Food Safety Authority (2015) Scientific opinion on the re-evaluation of sorbic acid (E 200), potassium sorbate (E 202) and calcium sorbate (E 203) as food additives. EFSA J 13(6):4144

Eurachem (2014) In: Magnusson B, Örnemark U (eds.) Eurachem guide: the fitness for purpose of analytical methods—a laboratory guide to method validation and related topics

Gatea F, Teodor ED, Paun G, Matei AO, Radu GL (2015) Capillary electrophoresis method validation for organic assessment in probiotics. Food Anal Methods 8:1335–1340

Holley RA, Millard GE (1980) Ultraviolet spectrophotometric determination of sorbic acid in dry fermented hot dog sausage. J AOAC Int 53:1332–1335

IARC International Agency for Research on Cancer (2015a) IARC monographs evaluate consumption of red meat and processed meat. Press Release No. 240. https://www.iarc.fr/en/media-centre/pr/2015/pdfs/pr240_E.pdf. Accessed 12 Jan 2016

IARC International Agency for Research on Cancer (2015b) Q&A on the carcinogenicity of the consumption of red meat and processed meat. http://www.iarc.fr/en/media-centre/iarcnews/pdf/Monographs-Q&A_Vol114.pdf. Accessed 12 Jan 2016

IBGE Instituto Brasileiro de Geografia e Estatística (2015) Estatística da produção pecuária. http://www.ibge.gov.br/home/estatistica/indicadores/agropecuaria/producaoagropecuaria/#animal. Acessed 20 Feb 2016

IUPAC International Union of Pure and Applied Chemistry (2002) Harmonized guidelines for single-laboratory validation of methods of analysis. Pure Appl Chem 74(5):835–855

Jaraith G, Singh PK, Dabur RS, Rani M, Chaudhari M (2015) Biogenic amines in meat and meat products and its public health significance: a review. J Food Sci Technol 52(11):6835–6846

Javanmardi F, Nemati M, Ansarin M, Arefhosseini SR (2015) Benzoic and sorbic acid in soft drink, milk, ketchup sauce and bread by dispersive liquid–liquid microextraction coupled with HPLC. Food Addit Contam 8:32–39

JECFA Joint FAO/WHO Committee on Food Additives (1974) Sorbic acid. http://www.fao.org/ag/agn/jecfa-additives/specs/Monograph1/Additive-430.pdf. Accessed 28 Sep 2014

Keeton J (2011) History of nitrite and nitrate in food. In: Bryan N, Loscalzo J (eds) Nitrite and nitrate in human health disease. Humana Press, New York, pp 69–84

Machado RMD, Tfouni SAV, Vitorino SHP, Vicente E, Toledo MCF (2007) Presença dos ácidos e sórbico em vinhos e sidras produzidos no Brasil. Cienc Tecnol Aliment 27(4):847–850

Mahboubifar M, Sobhani Z, Dehghanzadeh G, Javidnia K (2011) A comparison between UV spectrophotometer and high-performance liquid chromatography method for the analysis of sodium benzoate and potassium sorbate in food products. Food Anal Methods 4:150–154

Mihyar GF, Yousif AK, Yamani MI (1999) Determination of benzoic and sorbic acids in labaneh by high performance liquid chromatography. J Food Compos Anal 12:53–61

Nasser ALM, Lopes LMX, Eberlin MN, Monteiro M (2005) Identification of oligomers in polyethyleneterephthalate bottles for mineral water and fruit juice: development and validation of a high-performance liquid chromatographic method for the determination of first series cyclic trimer. J Chromatogr A 1097:130–137

OECD Organisation for Economic Cooperation and Development (2015) Meat consumption. Agricultural output. https://data.oecd.org/agroutput/meat-consumption.htm. Accessed 18 Apr 2016

Osawa T, Ishibashi H, Namiki M, Kada T, Tsuji K (1986) Desmutagenic action of food components on mutagens formed by the sorbic acid/nitrite reaction. Agric Biol Chem 50:1971–1977

Petruci JFS, Cardoso AA, Pereira EA (2011) Desenvolvimento e validação de método analítico para determinação de benzoato, sorbato, metil e propilparabenos em produtos alimentícios utilizando a eletroforese capilar. Quim Nova 34:1177–1181

Skoog DA, Holler FJ, Crouch SR (2006) Principles of instrumental analysis. Thomsom Brookes/Cole, California

Tfouni SAV, Toledo MCF (2002) Determination of benzoic and sorbic acid in Brazilian food. Food Contam 13:117–123

Ulca P, Atamer B, Keskin M, Senyuva HZ (2013) Sorbate and benzoate in Turkish retail foodstuffs. Food Addit Contam 6(3):209–213

Yu L, Wang S, Sun BG (2015) Food safety chemistry: toxicant occurrence, analysis and mitigation. CRC Press, Florida