



Contents lists available at ScienceDirect

Food Research International

journal homepage: [www.elsevier.com/locate/foodres](http://www.elsevier.com/locate/foodres)

## Screening and determination of aliphatic organic acids in commercial Brazilian sugarcane spirits employing a new method involving capillary electrophoresis and a semi-permanent adsorbed polymer coating

Mônia Stremel Azevedo<sup>a</sup>, Gabriela Pirassol<sup>a</sup>, Roseane Fett<sup>a</sup>, Gustavo A. Mücke<sup>b</sup>, Luciano Vitali<sup>a</sup>, Ana Carolina Oliveira Costa<sup>a,\*</sup>

<sup>a</sup> Department of Science and Food Technology, Federal University of Santa Catarina, Florianópolis, SC, Brazil

<sup>b</sup> Department of Chemistry, Federal University of Santa Catarina, Florianópolis, SC, Brazil

### ARTICLE INFO

#### Article history:

Received 10 June 2013

Received in revised form 28 October 2013

Accepted 6 November 2013

Available online xxxx

#### Keywords:

Aliphatic organic acids  
Brazilian sugarcane spirits  
Method validation  
Peakmaster® software

### ABSTRACT

A simple method for the screening and determination of short chain aliphatic carboxylic acids in Brazilian sugarcane spirits by indirect capillary zone electrophoresis was developed and validated according to Eurachem guidelines. The separations were performed in a coated capillary ( $L_{\text{tot}}$  73 cm  $\times$   $L_{\text{det}}$  64.5 cm) with 2-hydroxypropyltrimethyl-ammonium chloride chitosan. The background electrolyte was optimized using Peakmaster® software and was composed of 21 mmol L<sup>-1</sup>  $\beta$ -alanine and 10 mmol L<sup>-1</sup> 3,5 dinitrobenzoic acid, at pH 3.6, with an applied voltage of -30 kV. The method was validated for formic, lactic and acetic acids. Linearity was demonstrated and significant matrix effects were not observed at between 2.8 and 144 mg L<sup>-1</sup>. The recovery (means of 89.6–117.9%) was evaluated, and ANOVA was carried out to determine the RSD values for the repeatability (2.63–8.35%) and reproducibility (1.92–6.62%). The LOD and LOQ ranged from 0.69 to 2.70 and 2.76 to 8.11 mg L<sup>-1</sup>, respectively. The concentrations detected in the samples were 0.19–106.43 mg per 100 g.

© 2013 Elsevier Ltd. All rights reserved.

### 1. Introduction

Aliphatic organic acids are found in a wide range of foods and beverages (Klampfl, 2007). In the case of alcoholic beverages that are fermented or distilled, determination of the aliphatic organic acids allows control over the evolution of the acidity during the production steps (alcoholic fermentation, malolactic fermentation and aging process). In wine, acetic and lactic acids are associated with contamination by microorganisms and may be indicators of poor hygiene conditions (Sádecká, Májek, & Thóthová, 2008). The presence of aliphatic organic acids in the distillate is also related to the control of the processes occurring during the production and storage of the beverages. In sugarcane spirit (known in Brazil as *cachaça*), aliphatic organic acids originate directly from the distillation and/or fermentation of the sugarcane juice and they exert a strong influence on the quality of the sensory properties of this alcoholic beverage (Campos, Figueiredo, Hogg, & Couto, 2009). The Brazilian law establishes that aged sugarcane spirit should contain at least 50% of the spirit matured in appropriate wooden casks

(maximum capacity of 700 L) for a period of not less than 1 year. Premium and extra premium sugarcane spirits are spirits that were whole aged for 1 year and 3 years, respectively (Brasil, 2005). In aged alcoholic beverages, the concentration of aliphatic organic acids is directly related to the aging time and does not have a direct relationship with the aging process applied (Schawan, Mendonça, Silva, Rodrigues, & Wheals, 2001; Schwarz, Rodríguez, Guillén, & Barroso, 2011). Thus, the profile of these compounds can be used for the hygiene control, differentiation, classification, origin identification and/or detection of the adulteration of alcoholic beverages (Sádecká et al., 2008). This chemical monitoring contributes to the quality improvement and standardization of beverages such as sugarcane spirit, an alcoholic beverage widely consumed in Brazil and also appreciated on the international market (Nóbrega et al., 2009).

Major aliphatic organic acids previously detected in sugarcane spirit are: acetic, citric, formic, glycolic, lactic, maleic, malic, malonic, succinic, and tartaric (Chen, Lin, Chen, Misra, & Liu, 2003; Moreno, Jurado, & Barroso, 2003; Park, Kim, & Kim, 1999; Sádecká et al., 2008). Several methods to determine these aliphatic organic acids in food and beverages have been described in the literature based on different separation techniques including gas chromatography–mass spectrometry (GC–MS) (Gallagher et al., 2010; Jurado-Sánchez, Ballesteros, & Gallego, 2011), gas chromatography–flame ionization detection (GC–FID) (Ortega, Lopez, Cacho, & Ferreira, 2001; Park et al., 1999), high performance liquid chromatography–ultraviolet–visible detection (HPLC–UV/Vis) (Zhang et al., 2011), HPLC–conductivity detection (HPLC–CD)

**Abbreviations:** SCACA, short chain aliphatic carboxylic acids; CZE, capillary zone electrophoresis; EOF, electroosmotic flow; BGE, background electrolyte; HACC, 2-hydroxypropyltrimethyl-ammonium chloride chitosan; AOAC, Association of Official Analytical Chemists;  $R_s$ , resolution; I.S., internal standard;  $t_m$ , migration time; OLSM, ordinary least squares method;  $\alpha$ , significance level;  $L_{\text{tot}}$ , total length;  $L_{\text{det}}$ , effective length; TU, Tiselius unit;  $p$ , significance.

\* Corresponding author. Tel.: +55 48 3721 2975; fax: +55 48 3721 9943.

E-mail address: [ana.costa@ufsc.br](mailto:ana.costa@ufsc.br) (A.C.O. Costa).

(Schwarz et al., 2011), two-dimensional liquid chromatography using ion chromatography  $\times$  reversed-phase liquid chromatography (LC  $\times$  LC) (Brudin, Shellie, Haddad, & Schoenmakers, 2010), and capillary electrophoresis-UV/Vis (CE-UV/Vis) (Chen et al., 2003; Rovio, Kalliola, Siréna, & Tamminen, 2010; Sádecká et al., 2008). The technique of CE-UV/Vis offers the advantages of ease of application and reduced sample preparation time, allowing the high frequency provided by CE, good resolution and efficient separation of aliphatic organic acids, simple instrumentation, low consumption of chemicals, great potential for appropriate detection and quantification limits in food and beverage analysis, which are fundamental requirements in the development of analytical methods for quality control, and auxiliary software can be used for the rational optimization of buffer parameters for obtaining suitable separation of the analytes (Bianchi, Careri, & Corradini, 2005; Gaš, Jaroš, Hrušca, Zuskova, & Štědrý, 2005; Izco, Tormo, & Flores, 2002; Johns, Breadmore, Bruno, & Haddad, 2009; Mato, Suárez-Luque, & Huidobro, 2007; Santalad, Teerapornchaisit, Burakham, & Srijaranai, 2007). Anion separation by CE normally requires the use of an electroosmotic flow (EOF) inverter, which generates an anodic EOF to promote the separation in co-electroosmotic mode. This is required because in uncoated capillaries the anion separation occurs in counter-electroosmotic mode (cathodic EOF). In these capillaries, the EOF mobility varies over a wide range and is strongly dependent on the pH of the background electrolyte (BGE), which may adversely affect the separation of aliphatic organic acids with respect to time (long separation times) and simultaneity (anions with a higher or lower degree of mobility than the EOF) due to differences in terms of the mobility of these compounds. Capillary modifiers, such as tetradecyltrimethylammonium hydroxide (Mato et al., 2007; Moreno et al., 2003), cetyltrimethylammonium bromide (Esteves, Lima, Lima, & Duarte, 2004; Galli & Barbas, 2004; Rovio et al., 2010), tetraethylenepentamine (Fung & Lau, 2003) and hexadimethrine bromide (Bianchi et al., 2005) have been used. In most cases, these modifiers have been employed as additives in the BGE. However, hexadimethrine bromide can be used as a semi-permanent coating where the modifier is adsorbed on the surface of the fused silica capillary and does not need to be added to the BGE. The semi-permanent coating offers different advantages to other types of modifications, such as simplicity of coating formation, possibility of coating regeneration, access to *a priori* knowledge of the coating polymer properties, and reduced dependence of the coating process on surface chemistry (Lucy, MacDonald, & Gulcev, 2008). Furthermore, semi-permanent modifications can lead to a reduction in the baseline noise, compatibility with mass detectors, and no undesirable peaks in the electropherogram for the counter-ions, such as bromide, which is present in many modifiers and used as a BGE additive. A disadvantage in some cases is the low stability of the coating formed, for instance, hexadimethrine bromide resists for only ten runs. Thus, new polymeric modifiers need to be tested as polymers containing quaternized cationic groups for formation of the anodic EOF; for example, quaternary ammonium chitosan (2-hydroxy-propyltrimethyl-ammonium chloride chitosan; HACC) has been previously employed for the determination of anions and good results were obtained (Ma et al., 2007; Vitali, Horst, Heller, Fávere, & Micke, 2011).

The aim of this study was to develop a simple method for the screening and determination of 10 aliphatic organic acids in commercial Brazilian sugarcane spirits employing capillary zone electrophoresis (CZE) and using a semi-permanent capillary coating comprised of HACC. The development of the separation method, selection of the BGE compounds and instrumental parameters and evaluation of the dispersion effects of the analytes were performed using the Peakmaster® software (Gaš et al., 2005). The method was validated based on the evaluation parameters recommended in the Eurachem (1998) and Inmetro (2003) guidelines and then applied in the determination of acids previously identified in Brazilian sugarcane spirits: formic, lactic and acetic.

## 2. Materials and methods

### 2.1. Reagents and solutions

All chemicals used in the experiments were of analytical reagent grade. Short chain aliphatic carboxylic acids (maleic, malonic, tartaric, formic, citric, malic, glycolic, lactic, succinic, acetic), aspartic acid (internal standard – IS), 3,5-dinitrobenzoic acid, and  $\beta$ -alanine were obtained from Sigma-Aldrich (St. Louis, CO, USA). Deionized water with a resistivity of 18.2 M  $\Omega$  cm was obtained from a Milli-Q system (Millipore, Bedford, MA, USA) and used to prepare all solutions. HACC synthesized by Vitali et al. (2011) was kindly provided for the coating of the capillaries. A stock solution of 1.0% (w/v) HACC was prepared in 2 mmol L<sup>-1</sup> hydrochloric acid, filtered through a 45  $\mu$ m membrane and applied to modify the surface of the capillary. Standard stock solutions containing 1000 mg L<sup>-1</sup> of the aliphatic organic acids were prepared in deionized water. Calibration solutions were prepared by diluting the stock solutions with deionized water.

### 2.2. Capillary electrophoresis system and capillary coating

CE assays were conducted in a capillary electrophoresis system (model 7100, Agilent Technologies, Palo Alto, CA, USA), equipped with a diode array detector (set at 254 nm; indirect detection, with a reference at 360 nm for peak inversion), a temperature-control device (maintained at 25 °C), and data acquisition and treatment software supplied by the manufacturer (HP ChemStation, rev. A.06.01). The capillary was employed in separations and was coated by a procedure adapted from Vitali et al. (2011) using fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with dimensions of 73 cm total length, 64.5 cm effective length and 75  $\mu$ m inner diameter. The activation of the capillary surface by dissociation of the silanol groups was performed by flushing with 1 mol L<sup>-1</sup> NaOH for 30 min and then with deionized water for 15 min. After preconditioning, the capillary was modified by applying a semi-permanent coating of HACC through the following procedure: flush 10 min with HACC 0.2% (w/v), maintain in static contact for 10 min and, finally, flush the capillary for 5 min with BGE (without HACC). The reconstruction of the coating on different days was carried out by repeating these steps. The BGE used to determine the aliphatic organic acids was composed of 21 mmol L<sup>-1</sup>  $\beta$ -alanine and 10 mmol L<sup>-1</sup> 3,5-dinitrobenzoic acid, at pH 3.6. The standards and samples were introduced at the end of the capillary farthest from the detector and injected using a hydrodynamic pressure of 50 mbar for 3 s. The separation voltage applied was 30 kV, with negative polarity on the injection side. The capillary was flushed between runs for 1.0 min with BGE.

### 2.3. Samples

Samples of sugarcane spirit (with and without aging) were kindly provided by a local Brazilian sugarcane spirit industry production plant and the Brazilian Ministry of Agriculture and Livestock (MAPA): two non-aged samples (named 1-NA and 2-NA) and ten samples aged for 2 to 16 years (3-A2Y, 4-A2Y, 5-A3Y, 6-A3Y, 7-A3Y, 8-A4Y, 9-A5Y, 10-A6Y, 11-A12Y, 12-A16Y). Before injection into the CE equipment, the samples were diluted to give 9:1 (v/v) sample:I.S. solution (I.S. injected at 200 mg L<sup>-1</sup>).

### 2.4. Validation procedure and statistical analysis

The method was validated according to Eurachem (1998) and Inmetro (2003) guidelines employing assays with standard solutions, blank samples and spiked samples. System suitability, linearity, matrix effects, selectivity, precision, recovery, detection and quantification limits, and robustness were studied. The fitness-for-purpose of this method was assessed based on the results obtained for pre-

Download English Version:

<https://daneshyari.com/en/article/6396481>

Download Persian Version:

<https://daneshyari.com/article/6396481>

[Daneshyari.com](https://daneshyari.com)