Simultaneous voltammetric determination of four organic acids in fruit juices using multiway calibration

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ABSTRACT

A sensitive, fast, and inexpensive square wave voltammetric method using a cobalt phthalocyanine modified carbon paste electrode was developed for simultaneous determination of citric, lactic, malic and tartaric acids in fruit juices. To overcome the strong overlap of voltammetric signals caused by calibrated and uncalibrated constituents, multivariate curve resolution with alternating least squares (MCR-ALS) was used. Data were previously treated for correction of baseline and potential shift. The MCR-ALS calibration models were constructed and evaluated using a validation set obtained from a Taguchi design. The values predicted by the optimized MCR-ALS models were unbiased and no statistically significant difference was observed between proposed and reference methods, applying the paired *t*-test at a confidence level of 95%. As far as the authors know, a voltammetric method that simultaneously determines four organic acids in complex samples such as fruit juices without any previous pretreatment has not yet been reported in the literature.

1. Introduction

Organic acids such as citric, lactic, malic and tartaric are naturally found in fruit as result of metabolic processes, biochemical, hydrolysis and bacterial growth (Escobar et al., 2013; Flores, Hellín, & Fenoll, 2012). They play an essential role in the quality and nutritional value of food and contribute significantly to the organoleptic properties, microbiological and chemical stability of various foods and beverages, acting as antioxidant, emulsifier and acidity regulators (Gomis, Tamayo, Valles, & Alonso, 2004; Mato, Suárez-Luque, & Huidobro, 2005; Toaldo, Cruz, Silva, & Bordignon-Luiz, 2016). Moreover, the individual quantification these organic acids in fruit juice is very important for differentiation, classification, origin identification, or possible adulteration of beverages (Jurado-Sánchez, Ballesteros, & Gallego, 2011; Santalad, Teerapornchaisit, Burakham, & Srijaranai, 2007).

These organic acids have been quantified in various foods and beverages using laborious and complex spectrophotometric (Bureau et al., 2009), enzymatic (Oliveira, Lopes, Tóth, & Rangel, 2010), chromatographic (AOAC, 1990; Flores et al., 2012; Jurado-Sánchez et al., 2011; Kemmei, Kodama, Yamamoto, Inoue, & Hayakawa, 2015) and electrophoretic (Li et al., 2013) techniques. The Association Official of Analytical Chemistry (AOAC, 1990) has also been widely used to determine these organic acids (see Reference method section).

Simple, highly sensitive, fast and inexpensive voltammetric methods for quantifying these organic acids are unusual since they are not electroactives on common glass carbon, pyrolytic carbon and carbon paste electrodes. Modified electrodes using electron mediators have been employed to overcome this non electroactivity. Among the mediators most used are cobalt (II) phthalocyanines (CoPC) due to their actions as catalysts in redox processes that can occur quickly with small energy expenditure (Hosseini, Mahyari, Bagheri, & Shaabani, 2014) in either the central metal ion or the phthalocyanine ring (Kadish, Smith, & Guilard, 2003; Kozub & Compton, 2010; Rodríguez-Méndez et al, 2016; Rodríguez-Méndez, Gay, & Saja, 2009; Zagal, Griveau, Silva, Nyokong, & Bedioui, 2010). The electrocatalytic activity of the organic acids in CoPC-CPE involves the electrochemical oxidation of Co (II) to Co (III) followed by the chemical reduction of Co (III) to Co (II) by the organic acids in solution, as demonstrated by Nascimento et al. (2013) and Lourenço et al. (2018).

A pioneering work using CoPC-CPE for voltammetric determination of organic acids was proposed by Santos and Baldwin (1986). They developed a method for simultaneous quantification of oxalic and α-keto acids in urine. Later, CoPC-CPE was used for individual quantification of citric acid (Nascimento et al., 2013) in fruit juice, of lactic acid (Schmitt, Molitor, & Wu, 2012) in an aqueous solution and of tartaric acid (Lourenço et al., 2018) in wines. The simultaneous determination of citric, lactic, malic and tartaric acids in fruit juices, as far as the authors know, has not yet been reported in the literature.

Organic acids present electrocatalytic responses that potentially are very close to each other, resulting in a high degree of overlap among the voltammetric signals on CoPC-CPE (Schmitt et al., 2012). This overlap prevents voltammetric determination by univariate calibration when more than one organic acid is present in a sample. This drawback was overcome by Nascimento et al. (2013) using a slow and laborious sample pretreatment to eliminate the interference of ascorbic acid in the voltammetric determination of citric acid in orange juice, by using CoPC-PCE. For this purpose, the enzyme ascorbate oxidase was employed. Briefly, 20 mL of sample was stirred for 30 min in a glass vessel containing about 8 g of thin slices of cucumber mesocarp (Cucumis sativus) which is rich in ascorbate oxidase. Afterward, the sample was cleaned using a syringe filter with 0.45 mm porous membranes and properly diluted in the supporting electrolyte before analysis. A simpler, faster and less laborious way to overcome the overlapping of voltammetric signals is to use multiway calibration techniques (Lourenço et al., 2018).

The main characteristic of the multiway calibration is the "second order advantage" that enables one or more analytes to be accurately quantified in the presence of calibrated and uncalibrated constituents that are interfering (Arancibia, Damiani, Escandar, Ibañez, & Olivieri, 2012; Olivieri, 2012). Among the several multiway calibration methods available in the literature to model voltammetric data, multivariate curve resolution with alternating least squares (MCR-ALS) is distinctive due to the flexibility of the MCR-ALS algorithm to model a data tensor where the break in trilinearity occurs. This problem arises when one of the data modes is nonreproducible. For example, the frequency mode is not reproducible among samples (Jalalvand & Goicoechea, 2017; Jalalvand, Goicoechea, & Rutledge, 2017; Olivieri, Escandar, Goicoechea, & Peña, 2015).

This present work presents for the first time a highly sensitive method for simultaneous square wave voltammetric determination of citric (CA), lactic (LA), malic (MA) and tartaric (TA) acids in fruit juice samples using CoPC-CPE. To overcome overlapping signals caused by calibrated and uncalibrated constituents, MCR-ALS was used. As far as we know, the electrocatalytic oxidation study and the simultaneous voltammetric determination of CA, LA, MA and TA in fruit juice samples have not been reported in the literature yet.

2. Experimental

2.1. Chemicals

All chemicals were of analytical-reagent grade and used as received. All solutions were prepared with deionized water from a Millipore Milli-Q system (conductivity $\leq 0.1\,\mu S\,cm^{-1}$). Acetic acid and sodium acetate obtained respectively from J.T. Baker and Sigma-Aldrich were used to prepare the $0.05\,mol\,L^{-1}$ acetate buffer. CoPC (97%), mineral oil and graphite powder ($\leq 20\,\mu m$) were acquired from Sigma-Aldrich. The citric, lactic, malic, succinic and tartaric acids were purchased from VETEC and stock solutions of each organic acid ($0.0125\,mol\,L^{-1}$) were prepared daily in the supporting electrolyte (acetate buffer adjusted to pH = 3.5, 4.5 and 6.5).

2.2. Calibration set

Ten individual standard solutions of each calibrated constituent (organic acids: CA, LA, MA and TA) with concentrations ranging from 10 to $100\,\mu mol\,L^{-1}$ were prepared in an acetate buffer (pH 4.5) by direct dilution within the electrochemical cell, and used as the calibration set to build the MCR-ALS models.

2.3. Validation set

A validation set (Table S1 at the Supplementary Material) containing twenty-five mixtures of calibrated constituents (organic acids:

CA, LA, MA and TA) and an uncalibrated constituent (organic acid: succinic acid (SA)) were prepared according to a Taguchi design.

SA was added as an uncalibrated constituent in the validation set to mimic a possible interference scenario for quantification of calibrated constituents in order to assess whether the MCR-ALS was able to quantify the calibrated constituents in the presence of an uncalibrated constituent. This property is known as the second order advantage of the multiway calibration (as the MCR-ALS) (Olivieri and Escandar, 2014).

Taguchi design was chosen because it uses special set orthogonal arrays which make it possible to find the best values of the controllable factors with less sensitivity to the variations in the uncontrollable factors and with the fewest number of experiments (Cavazzuti, 2013; Gaitonde, Karnik, Achyutha, & Siddeswarappa, 2006; Kacker, Lagergren, & Filliben, 1991).

2.4. Fruit juice samples

Nine samples of fruit juices previously analized by the reference method (AOAC, 1990) were provided from the laboratory LABV/SFA/LANAGRO-PE (Laboratório Nacional Agropecuário em Pernambuco – Base Física II – Laboratório de Análises Físico-Químicas de Bebidas e Vinagres").

The following volumes of each fruit juice: 50 μL of lemon, orange or guava; 10 μL of cashew, passion fruit, cajá or acerola; and 100 μL açaí + guarana were added to an electrochemical cell containing 20 mL of acetate buffer (pH 4.5) and their SW voltammograms were recorded. Different volumes of each fruit juice were chosen, taking into account that the maximum voltammetric signal of each sample was within the linear working range of CA, LA, MA and TA, as can be seen in Fig. 3. It is worth noting that a 100 μL volume was used in açaí + guarana analysis because the concentrations of CA, LA, MA and TA found in this fruit juice are lower than those obtained in the other fruit juices analyzed, as can be seen in Table 3. These samples were always analyzed without any previous treatment.

2.5. Reference method

The reversed phase High Performance Liquid Chromatography (HPLC) coupled with UV–Vis detection of AOAC was used as the reference method (AOAC, 1990). This slow and laborious method requires the following steps: extraction of the organic acids with organic solvent (ethyl acetate), decantation with anhydrous sodium sulfate (duration of 30 min), evaporation to < 25 mL in a steam bath under a gentle stream of clean nitrogen; cooling to room temperature, adjusting the volume to 25.0 mL with fresh ethyl acetate, filtration through a syringe-tip filter (0.45 μm pores) prior to injection and finally the sample injection into the HPLC containing two C18 columns positioned in series.

2.6. Apparatus

All voltammetric measurements were performed using an Eco Chemie potentiostat/galvanostat Autolab (Model PGSTAT 302N) controlled by NOVA 1.10.4 software and a system of three electrodes: the reference electrode, Ag/AgCl (KCl, $3\,mol\,L^{-1}$); an auxiliary electrode, platinum wire, and unmodified paste; and cobalt (II) phthalocyanine-modified carbon paste electrodes as the working electrodes.

The unmodified carbon paste electrode was prepared by homogenizing carbon powder and mineral oil (60/40% m/m). The cobalt (II) phthalocyanine (CoPC) modified carbon paste electrode (CoPC-CPE) was prepared by homogenizing carbon powder with CoPC (57/3% m/m). Then, the carbon powder and the CoPC (carbon/CoPC) mixture were dispersed in acetone under stirring until complete evaporation of the solvent. Subsequently, mineral oil was added to the carbon/CoPC mixture (40/60% m/m). Finally, the paste was packed into a glass tube