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Short communication

Simple and fast method for simultaneous determination of propionate and sorbate in bread by capillary electrophoresis with UV spectrophotometric detection

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ABSTRACT

A novel method for simultaneous determination of the preservatives propionate and sorbate in bread samples using capillary electrophoresis with diode array spectrophotometric detection is proposed. This method requires a simple and fast sample preparation using aqueous extraction with sonication aid. A background electrolyte (BGE) composed of 10 mmol L^{-1} benzoic acid, 15 mmol L^{-1} L-histidine and 0.2 mmol L^{-1} of cetyltrimethylammonium bromide (CTAB) allowed the separation of the preservatives with good resolution in less than 5 min. Propionate was indirectly detected at 235 nm, and the sorbate was directly detected at 250 nm. The limits of quantification (LOQ) were 4.3 and 1.5 mg kg^{-1} for propionate and sorbate, respectively. The proposed method was successfully applied for analysis of commercial samples of sliced white bread.

1. Introduction

Propionic (pK_a = 4.87) and sorbic (pK_a = 4.76) acids and their salts (sodium, potassium, and calcium) are widely used as antimicrobial agents in the food industry. These compounds inhibit the growth of the population of bacteria, yeast, and fungi in foods because these microbials are unable to metabolize the molecules of the preservatives (Carocho et al., 2014; Smith et al., 2004). So, the addition of sorbates and propionates in foods avoids biological deterioration and extends their shelf-life. Propionates and sorbates are of low human toxicity and are extensively metabolised in the human body. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established an acceptable daily intake (ADI) of sorbates (expressed as sorbic acid) as 25 mg/kg/day (FAO/WHO, 1962). The European Food Safety Authority (EFSA) in 2015 re-evaluated the sorbates as food additives and reduced the recommended ADI from 25 to 3 mg/kg/day for sorbic acid and its potassium salt (EFSA, 2015). In contrast, it was considered unnecessary to establish a recommended ADI for propionates.

Only a few works have reported analytical methods for the simultaneous determination of propionate and sorbate in foods (Beljaars et al., 1996; Chikamoto et al., 1988; Chu et al., 1986; Guo et al., 2012; Kaufmann et al., 2018; Lou et al., 2017; Petroturza et al., 1980). Gas chromatography (GC) is the most used analytical technique for determination of these preservatives in food, mainly propionate (Abedi et al., 2014; Ibanez, 2003; Sasaki et al., 2016; Wang et al., 2006; Yang et al., 2012; Yang and Choong, 2001). However, these GC methods usually require laborious and time-consuming derivatisation of the analytes, prior to analysis. Therefore, there is a demand for the development of alternative analytical methods for rapid and easy quantitative determination of sorbate and propionate, the compounds most used as antimicrobial preservatives to prevent spoilage of bread, particularly by moulds (Marin et al., 2002). The Brazilian regulatory agency for the approval and supervision of foods (ANVISA) established that the amount of sorbate added to bread must be lower or equal to 0.1% (*w/w*) or 1 g kg^{-1} (Anvisa (1999)). Propionate addition is unlimited, and its typical concentration in this kind of foodstuff is in the range of 0.1–0.5% (w/w) or 1–5 g kg^{-1}. In the literature, a limited number of works using GC (Beljaars et al., 1996), HPLC (Javanmardi et al., 2015), capillary electrophoresis (CE) (Ackermans et al., 1992), and colorimetry (Phechkrajang and Yooyong, 2017) report the determination of propionate and sorbate in bread. Nevertheless, only in the GC method was a simultaneous determination of both preservatives performed.

CE is an interesting technique for simultaneous determination of propionate and sorbate because depending on the pH of the aqueous

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solution both compounds can be in the ionic form (electrically charged). Direct photometric detection of sorbate is feasible as this preservative shows strong absorption of UV radiation (250 nm). In contrast, indirect detection by adding a chromophore agent in the background electrolyte (BGE) is required for propionate detection because it shows low absorptivity in the UV–vis range. Ackermans et al. (1992) used this approach for determination of propionate in bread by a CE method that used benzoic acid in the BGE as a chromophore agent. Guo et al. (2012) determined sorbic acid and propionate in beverages, cake, and biscuit by CE with indirect laser-induced fluorescence using cadmium telluride quantum dots as fluorescent labels.

In this work, CE was used as a rapid and simple analytical technique for simultaneous determination of propionate and sorbate in samples of commercial sliced bread. The sample preparation was simple, comprising only an aqueous extraction of the preservatives with sonication. To the best of our knowledge, this work is the first that used CE-UV for simultaneous determination of propionate and sorbate in bread.

2. Material and methods

2.1. Reagents

All the reagents were of analytical grade. Methanol, NaOH, and HCl were purchased from Labsynth (Diadema, SP, Brazil). Sodium acetate, calcium propionate, potassium sorbate, L-histidine, benzoic acid, and cetyltrimethylammonium bromide (CTAB) were from Sigma-Aldrich (Steinheim, Germany). Ultrapure water was obtained from a Direct-Q 3 UV water purification system (Millipore, Molsheim, France).

2.2. Standard solutions and samples

Stock solutions of potassium sorbate and calcium propionate were prepared by dissolving amounts of the solid reagents in ultrapure water to achieve a final concentration of 1.00 g L^{-1} in sorbate and propionate. By dilution of these stock solutions, working standard solutions containing both preservatives (0.083–100 mg L^{-1} for propionate and 0.03–10 mg L^{-1} for sorbate) were prepared to obtain calibration curves. Acetate was added (10 mg L^{-1}) to all solutions as an internal standard.

Four different samples of commercial sliced white bread were acquired at local markets in the city of Campinas, São Paulo state, Brazil. The bread samples were purchased in packages containing 500 g (about 15–20 slices). Five slices of each bread sample were put inside sealed plastic bags that were then stored in a refrigerator (4 °C) for up to a week.

2.3. CE analysis

All separations were conducted using an Agilent 7100 CE system (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector (DAD). Bare fused silica capillary with 75 μm i.d. with a total length of 75 cm (67 cm effective) was used. The BGE was composed of 10 mmoL L^{-1} benzoic acid and 15 mmol L^{-1} L-histidine (His), resulting in a pH of 5.8. CTAB was added (0.2 mmol L^{-1}) to the BGE as electroosmotic flow (EOF) inverter. Capillary column conditioning was daily conducted by sequential flushes with methanol (5 min), 1 mol L^{-1} HCl (5 min), H_2O (3 min), 1 mol L^{-1} NaOH (5 min), H_2O (3 min), and BGE (5 min). Hydrodynamic injection for 5 s at 50 mbar was performed. Between the runs, the capillary was flushed with 0.1 mol L^{-1} NaOH (1 min), H_2O (0.5 min), and BGE (1 min) to avoid EOF variation, particularly when the samples were injected. The separation voltage was −25 kV and the spectrophotometric detection was conducted at 235 and 250 nm for propionate and sorbate, respectively.

2.4. Sample preparation

A slice of bread sample was cut into small pieces (cubes of about 3–4 mm) using a knife. In the following, 1 g of bread was weighed (with an accuracy of 0.1 mg) and transferred to a 100-mL glass Erlenmeyer flask. Ultrapure water at a volume of 50 mL was added to the flask. The suspension formed was sonicated for 10 min, and about 5 mL of the extract were filtered using a polyvinylidene difluoride (PVDF) membrane filter (0.2 μm). Finally, the filtered aqueous extract was 2-fold diluted with ultrapure water, the internal standard acetate was added (10 mg L^{-1}), and injected into the CE system.

2.5. Recovery tests

The accuracy of the proposed method was evaluated by recovery tests that were performed for all commercial samples at three concentration levels. Briefly, the sample preparation was conducted as already described, but before the sonication procedure, the aqueous extracts were spiked with known concentrations of the preservatives.

3. Results and discussion

3.1. CE separation

Fig. 1A,B shows electropherograms of a standard solution of sorbate, propionate, and acetate (internal standard) and for an aqueous extract obtained from a bread sample. Separations with good peak resolutions were achieved in less than 5 min. Acetate was chosen as a suitable internal standard (IS) because this compound was absent in the bread samples, and its electrophoretic mobility is close to those of the preservatives.

In the bakery industry, lactic acid is commonly added to bread to control the acidity of this foodstuff. Lactic acid (as lactate) has electrophoretic mobility close to that of propionate, so an appropriate choice of the pH of the BGE was necessary to avoid co-migration of these ions. BGE with pH higher (6.0, 6.2, and 6.5) or lower (5.6, 5.5, and 5.3) than 5.8 led to unsatisfactory resolutions between the lactate and propionate peaks. Thus, the BGE with pH of 5.8 was chosen because it provided a baseline separation of the preservative and lactate, as depicted in Fig. 1B.

The DAD allowed simultaneous detection of the preservatives at two different wavelengths (235 and 250 nm). As propionate and acetate lack a strong chromophore group, benzoic acid was added to the BGE to increase the background UV absorption, which allowed an indirect detection (negative peaks) of these compounds at 235 nm. In contrast, sorbate could be directly detected (positive peak) because this preservative has a higher absorbance at 250 nm than that of the BGE.

3.2. Figures of merit of the method

The main analytical parameters of the proposed method were evaluated and are summarised in Table 1. The relative standard deviation (RSD) for the migration times of the peaks was 1.3% for propionate and 1.9% for sorbate. The high number of plates (N/m) is compatible with the high separation efficiency commonly obtained by CE. The values of the coefficients of determination (r^2) higher than 0.999 for the linear regressions of the calibration curves indicated good linearity. To confirm this linearity, ANOVA lack-of-fit test (Araujo, 2009; Van Loco et al., 2002) was performed. As the p-values obtained for the lack-of-fit test (Table 1) were higher than 0.05 (confidence level of 95%), the linear regression model was considered adequate.

The instrumental limits of detection (LOD_i) and quantification (LOQ_i) were calculated as the concentrations of the aqueous extracts that yield signal-to-noise ratios (S/N) equal to 3 and 10, respectively. LOD_m and LOQ_m are the limits of detection and quantification of the method, respectively, calculated considering the mass of bread (1 g)