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Analytical Methods

Quality control of fruit juices by using organic acids determined by capillary zone electrophoresis with poly(vinyl alcohol)-coated bubble cell capillaries

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ABSTRACT

An enhanced method for the determination of organic acids in several fruit juices by capillary zone electrophoresis (CZE) with direct UV–Vis detection has been developed in this work. First, a study with simulated real juice samples was done to find the best separation conditions. Next, several commercial fruit juices were analyzed, and the organic acid contents were quantified in less than 12 min using a poly(vinyl alcohol)-coated fused-silica 'bubble cell' capillary. The present method is reliable, fast and provides detection limits comprised between 0.1 and 2.5 μg mL^{-1}. Moreover, different chemometric techniques, based on CZE data, were examined. Linear discriminant analysis allowed the differentiation of fruit juices according to the fruit type, whereas multiple linear regression models predicted the percentages of orange and pineapple juices in binary blends with grape. Thus, the present methodology is of utmost interest for routine and quality control purposes in food industries.

1. Introduction

The fruit juice industry is one of the fastest growing sectors of the worldwide beverage industry. Fruit juices, which are widely consumed, are an important part of the human diet, and have become very popular due to their many reported health benefits (essential vitamin and mineral content, aid in the prevention of cancer, aid digestion, anti-inflammatory properties, increase bone strength, etc.) (Jandric et al., 2014). The economic value of fruit juices makes the product disposed to adulteration, which has a negative impact not only on the consumer, which expect that manufacturers and retailers provide authentic fruit juices, but also on the industry, where quality authentic products have to compete with less expensive adulterated products, having also the responsibility to comply with labeling legislation (Stander, Kühn, & Hiten, 2013). In fact, the fruit juice industry Code of Practice published by the AIJN (Association of the industry of juices and nectars from fruits and vegetables) has provided guidelines for general fruit authenticity and quality criteria (AIJN, 2010).

To date, the most frequently methods used to detect fruit juice adulteration are based on the profiling and quantification of a number of compounds that may be from one chemical family or from different families (Jandric et al., 2014), such as carbohydrates (Kelebek, Selli, Canbas, & Cabaroglu, 2009; Muntean, 2010; Stander et al., 2013), phenolic compounds (Díaz-García, Obón, Castellar, Collado, & Alacid, 2013; Kelebek et al., 2009; Obón, Díaz-García, & Castellar, 2011;Stander et al., 2013), amino acids (Gómez-Ariza, Villegas-Portero, & Bernal-Daza, 2005; Simó, Rizzi, Barbas, & Cifuentes, 2005; Tezcan, Uzaşçi, Uyar, Öztekin, & Erim, 2013), anthocyanins and pigments (Obón et al., 2011) and organic acids (Ehling & Cole, 2011; Kelebek et al., 2009; Mato, Huidobro, Simal-Lozano, & Sancho, 2006; Saavedra, García, & Barbas, 2000; Saavedra, Rupérez, & Barbas, 2001; Scherer et al., 2012; Tezcan, Gültekin-Özgüven, Diken, Özçelik, & Erim, 2009), among others.

Separation, identification and quantification of the major organic acids present in a fruit juice is of considerable importance, since their presence and relative ratio can affect the chemical and organoleptic characteristics of the juice (e.g., pH, total acidity, global acceptability) (Chinnici, Spinabelli, Riponi, & Amati, 2005), providing also useful information not only about its authenticity, but also about microbiological alterations that may have occurred previously (Cunha & Fernandes, 2002).

Fruit juices have distinct organic acid profiles that can be used as fingerprints for establishing authenticity (Cordella, Moussa, Martel, Sbirrazzouli, & Lizzani-Cuvelier, 2002; Ehling & Cole,

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2011; Kvasnicka, 2005; Saavedra et al., 2000; Shui & Leong, 2002). Tartaric acid is usually considered an indicator of grape juice addition to more expensive juices (AIJN, 2010; Ehling & Cole, 2011; Saavedra et al., 2000). Isocitric acid, which is present at much lower concentrations than other organic acids, is also too expensive to be added with adulteration purposes (Sadecka, Polonsky, Simko, & Karasova, 2001). Thus, it could be used to evaluate authenticity and quality of citrus juices (Jezek & Suhaj, 2001; Kvasnicka, Voldrich, Pys, & Vins, 2002). According to the AIJN (2010), in reconstituted orange juice of 11.2° Brix the content of isocitric acid is between 65 and 200 μg mL^{-1}. The ratio of citric acid to isocitric acid has also been used to establish a chemical profile of authentic fruit juices. For example, the adulteration of orange concentrate or juice by addition of sugars, citric acid and water can be detected from this ratio, which is usually lower than 130 in authentic orange juice (AIJN, 2010).

Different techniques have been employed for organic acid determination in fruit juices, mainly enzymatic (Stój & Targonski, 2006) and chromatographic (Chinnici et al., 2005; Cunha & Fernandes, 2002; Ehling & Cole, 2011; Kelebek et al., 2009; Scherer et al., 2012; Shui & Leong, 2002) methods. However, enzymatic analysis require specific kits for each organic acid, they are time-consuming and use large amounts of reagents, which make them expensive. On the other hand, chromatographic methods, such as traditional HPLC, usually require purification techniques to eliminate matrix interferences (e.g., sugars or phenolic compounds), which has a negative influence on the simplicity and quickness of the method, especially for routine quality control analysis. Then, other techniques, such as capillary electrophoresis (CE), have been also described (Jezek & Suhaj, 2001; Mato et al., 2006; Saavedra et al., 2000; Sadecka et al., 2001). CE has proved to be a good choice for the determination of samples in aqueous media, since usually no more than a simple dilution of samples is needed. This technique has been used by Saavedra et al. (2000) for the direct measurement of malic, tartaric, isocitric and citric acids as adulteration markers in orange juices. However, other fruit juice matrices (apple, grape, pineapple, mandarin, etc.) were not determined. Thus, and in order to cover this demand, it is necessary to achieve the main organic acid patterns typically found in different fruit juices, and to study their implication in quality control purposes.

In this work, a reliable and sensitive CZE method using direct UV detection for the determination of organic acids in several fruit juices was described. For this purpose, an optimization study with simulated real juice samples (containing large variability of organic acid contents) using different experimental CZE conditions (including poly(vinyl alcohol) (PVA)-coated capillaries with normal and with extended-path light was first carried out. The organic acid content present in different fruit juices was obtained. Next, a linear discriminant analysis (LDA) model was constructed to classify juices according to the type of fruit employed and to distinguish between pure juices and juice blends. Moreover, data treatment by multiple linear regression (MLR) was used to quantify blends of high-value juices (pineapple and orange) with grape juice.

2. Material and methods

2.1. Chemicals and samples

The following analytical grade reagents were used: sodium hydroxide (NaOH) and sodium dihydrogenphosphate (NaH_2PO_4) (Sigma–Aldrich, St. Louis, MO). Deionized water (Barnstead deionizer, Sybron, Boston, MA) was also used. The analytical standards were: oxalic (internal standard, IS), fumaric, isocitric, malic, tartaric and citric acids (Sigma–Aldrich). Individual stock solutions of organic acids were prepared in water at 10,000 μg mL^{-1}, except for isocitric and fumaric acids, which were 1000 and 100 μg mL^{-1}, respectively. The 30 fruit juices employed in this study, 6 for each type of fruit (see Supplementary material, Table S1) were purchased from the Spanish market.

To evaluate the possibility of detecting blends of fruit juices, binary mixtures containing different percentages of pineapple–grape and orange–grape juices were prepared. To improve the robustness of the MLR models, the mixtures were prepared using all the available fruit juices, which were randomly selected and mixed. For instance, for the pineapple–grape mixture, a total of 44 mixtures, four for each different percentage, which comprised between 0% and 100% pineapple, were performed. One more set of 44 mixtures containing the same percentages of orange juice was also prepared for the orange–grape mixture. Thus, a total of 88 mixtures were made.

2.2. Instrumentation and procedures

An HP^{3D} CE system (Agilent, Waldbronn, Germany) provided with a diode array spectrophotometric detector was adopted. Two different fused-silica capillaries were used: a PVA-coated with normal path light and a PVA-coated with extended path light (“bubble cell”), both with 64.5 cm length (56 effective length) × 50 μm id (375 μm o.d.) (Agilent). Between runs, the capillary was flushed with the BGE for 4 min. The BGE solutions used were prepared by dissolving an appropriate amount of NaH_2PO_4 to obtain a final phosphate concentration of 200 mM, and the pH values were adjusted by adding the required amount of NaOH or HCl 1 M. Before injection, all solutions were filtered through 0.45 μm pore size nylon filters (Albet, Barcelona, Spain). The optimal CZE conditions employed in this work were: BGE containing 200 mM phosphate buffer at pH 7.5, separation −15 kV (current −125 μA) at 25 °C, hydrodynamic injection (50 mbar × 20 s) and UV signal at 200 ± 20 nm (450 ± 80 nm as reference). Data acquisition was performed with ChemStation Software (Rev.A.10.01, Agilent). Statistical data treatment was performed using SPSS (v. 15.0, Statistical Package for the Social Sciences, Chicago, IL).

2.3. Sample preparation

Fruit juices, previously refrigerated at 5 °C, were centrifuged at 10,000 rpm for 10 min. The supernatant was 1:5 (v/v) diluted with deionized water, and for quantification purposes, the IS at 150 μg mL^{-1} was also added. However, for apple juices, quantification was also performed using a 1:1 (v/v) dilution due to the low content of fumaric acid in these samples (<5 μg mL^{-1}) (AIJN, 2010). The samples were analyzed in triplicate.

3. Results and discussion

3.1. Establishment of CZE method using simulated juice samples

In order to achieve organic acid separation, test mixtures containing the standards at different concentration levels and the IS described in Section 2.3 were used. The initial BGE and separation conditions were adapted from the work of Saavedra et al. (2000), which used a Beckman neutral polyacrylamide coated capillary. Thus, a BGE containing 200 mM phosphate at pH 7.5 (at −15 kV and 25 °C) was tested in this study. However, in this work, a commercial PVA-coated capillary, instead of the Beckman polyacrylamide coating one, was employed. These conditions were applied to the analysis of two different standard mixtures: (i) a mixture containing 150 μg mL^{-1} of malic, tartaric, isocitric, citric and fumaric acids, and (ii) a mixture that simulated a real