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Merging a sensitive capillary electrophoresis–ultraviolet detection method with chemometric exploratory data analysis for the determination of phenolic acids and subsequent characterization of avocado fruit

Elena Hurtado-Fernández, Paulina K. Contreras-Gutiérrez, Luis Cuadros-Rodríguez, Alegría Carrasco-Pancorbo *, Alberto Fernández-Gutiérrez

Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Ave. Fuentenueva s/n, E-18071 Granada, Spain

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ABSTRACT

Herein we present the development of a powerful CE–UV method able to detect and quantify an important number of phenolic acids in 13 varieties of avocado fruits at 2 ripening stages. All the variables involved in CE separation were exhaustively optimized and the best results were obtained with a capillary of 50 μm i.d. × 50 cm effective length, sodium tetraborate 40 mM at a pH of 9.4, 30 kV, 25 °C, 10 s of hydrodynamic injection (0.5 psi) and UV detection at 254 nm. This optimal methodology was fully validated and then applied to different avocado samples. The number of phenolic acids determined varied from 8 to 14 compounds; in general, they were in concentrations ranging from 0.13 ppm to 3.82 ppm, except *p*-coumaric, benzoic and protocatechuic acids, which were found at higher concentrations. Principal component analysis (PCA) was applied to highlight the differences between varieties and ripening degrees, looking for the most influential analytes.

1. Introduction

Avocado (*Persea americana*) is a tropical or sub-tropical fruit native to South America, but it is widely cultivated throughout the world (Ozdemir & Topuz, 2004). In fact, there are extensive crops of avocado in the coasts of Granada and Málaga (located in the south of Spain). According to data for 2010 of the Food and Agriculture Organization of the United Nations (FAOSTAT, 2010), the harvested area in Spain have increased around 500 Ha since 2005 and Spain occupies the 11th position on the world list of avocado production, with Mexico as number one.

The consumption of avocado fruit is increasing because of the numerous healthy benefits that it possesses, which is related to its composition (Ding, Chin, Kinghorn, & D´Ambrosio, 2007; Landahl, Meyer, & Terry, 2009; Meyer & Terry, 2010). This fruit is considered an important source of energy (Ozdemir & Topuz, 2004; Takenaga, Matsuyama, Abe, Torii, & Itoh, 2008), mainly for its high amounts of lipids (Hierro, Tomás, Fernández-Martín, & Santa-María, 1992); specifically avocado is rich in monounsaturated fatty acids, such as oleic acid; the main fatty acid (Gaydou, Lozano, & Ratovohery, 1987; Pacetti, Boselli, Lucci, & Frega, 2007). It also contains carbohydrates (C7); proteins; dietary fibre; vitamins E, C, B_2, B_5 and B_6; potassium; magnesium and phosphorus (Slater, Shankman, Sheperd, & Alfin-Slater, 1975; Bergh, 1992; Whiley, Schaffer, & Wolstenholme, 2002). Moreover, it has considerable amounts of pigments [carotenoids (α-carotene, β-carotene, cryptoxanthin, lutein, isolutein, zeaxanthin, etc.) (Gross, Gabai, Liefshitz, & Sklarz, 1973), chlorophylls (chlorophylls a and b) and anthocyanins (cyanidin 3-*O*-glucoside) (Ashton et al., 2006)]; sterols (β-sitosterol, campesterol, stigmasterol) (Duester, 2001; Piironen, Toivo, Puupponen-Pimiä, & Lampi, 2003; Plaza, Sánchez-Moreno, de Pascual-Teresa, de Ancos, & Cano, 2009); and phenolic compounds (phenolic acids and some flavonoids) (Golan, Kahn, & Sadovski, 1977; Van Lelyveld, Gerrish, & Dixon, 1984; Torres, Mau-Lastovicka, & Rezaaiyan, 1987; Terasawa, Sakakibara, & Murata, 2006; Golukcu & Ozdemir, 2010; Gorinstein et al., 2010; Poovarodom et al., 2010).

Phenolic acids are secondary metabolites present in plants, usually conjugated to sugars or to other molecules as proteins, cellulose or lignins; and they constitute a big class of compounds included into the group of phenolic compounds (Mattila, Hellström, & Törrönen, 2006; Hounsome, Hounsome, Tomos, & Edwards-Jones, 2008). According to its origin, phenolic acids can be classified as derived from benzoic acid or from cinnamic acid (Mattila & Kumpulainen, 2002; Stalikas, 2007). These compounds

* Corresponding author. Tel.: +34 958 249584; fax: +34 958 243328.
E-mail address: alegriac@ugr.es (A. Carrasco-Pancorbo).

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are related to different functions in plants (Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999; Kubán, Sterbová, & Kubán, 2006); besides they have been associated with colour and organoleptic properties of foodstuffs and the food industry has investigated the effect of the phenolic acids on fruit maturation and their roles as food preservatives (Shahidi & Nacsk, 1995). Moreover, the relationship between the intake of phenolic acids and its health benefits has been extensively described in literature (Fukuji, Tonin, & Tavares, 2010), since they have antioxidant properties and antibacterial, anticarcinogenic and anti-inflamatory activities, among others (Mattila et al., 2006; Mattila & Hellström, 2007).

Both phenolic compounds (Naczk & Shahidi, 2006; Proestos, Sereli, & Komaitis, 2006; Helmja, Vaher, Püssa, Raudsepp, & Kaljurand, 2008) and phenolic acids (Mattila & Kumpulainen, 2002; Stalikas, 2007; Fukuji et al., 2010) have been traditionally determined in plant matrices by using different separative techniques coupled to diverse detection systems. Liquid chromatography is normally the technique of choice (Mattila & Kumpulainen, 2002; Stalikas, 2007), because it offers a wide range of advantages. At the end of the 1990s, CE started to receive increased attention (Carrasco-Pancorbo, Cruces-Blanco, Segura-Carretero, & Fernández-Gutiérrez, 2004; Fukuji et al., 2010), because it is a powerful tool that provides high efficiency, short analysis time, versatility and the consumption of solvent is low (Carrasco-Pancorbo, Neusüb, Pelzing, Segura-Carretero, & Fernández-Gutiérrez, 2007; Hurtado-Fernández, Gómez-Romero, Carrasco-Pancorbo, & Fernández-Gutiérrez, 2010). However, after this promising start, some analysts found that CE did not achieve the expectations that the scientific community had. Although it is quite clear that the reproducibility and the sensitivity are the two the main problems of CE, there are some ways to solve these drawbacks and there are interesting applications in which this technique can get very valuable results.

The objective of this study was to demonstrate the usefulness of CE to identify and quantify the phenolic acids present in the pulp of different avocado fruit. Principal component analysis (PCA) was applied to highlight the differences between varieties and ripening degrees. To the best of our knowledge, this is the first time in which a CE–UV method together with statistical tools has been used for the determination of phenolic acid profile and analytical characterization of *P. americana*.

2. Material and methods

2.1. Chemicals and standards

Standards of phenolic acids (vanillic, homovanillic, isovanillic, ferulic, 4-hydroxybenzoic, benzoic, *trans*-cinnamic, syringic, caffeic, gallic, gentisic, *p*-coumaric, *m*-coumaric, *o*-coumaric, protocatechuic, *o*-pyrocatechuic, sinapic, chlorogenic, α-resorcylic, β-resorcylic, γ-resorcylic acids) were purchased from Extrasynthese (Lyon, France), Sigma–Aldrich (St. Louis, EEUU) and Fluka (St. Louis, EEUU); its structures are shown in Fig. 1. The stock solution containing the 21 compounds was prepared in methanol/water (50:50, v/v) at a concentration of 9.5 ppm.

Taxifolin was purchased from Extrasynthese (Lyon, France) and it was used as an internal standard (IS) to evaluate the reproducibility of the extraction system and the electrophoretic procedure.

Methanol, used as a solvent for the sample extraction and the preparation of stock solutions, was purchased from Panreac (Barcelona, Spain). Hydrochloride acid was acquired from VWR (West Chester, EEUU) and it was used, together with sodium hydroxide (from Panreac), to adjust the pH of running buffers. Sodium tetraborate (borax), sodium carbonate anhydrous and 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO) were obtained from Sigma–Aldrich (St. Louis, EEUU) and from Panreac (Barcelona, Spain); all of them were used as running buffers at different concentrations and pH values. All the solvents were HPLC grade and they were used as received.

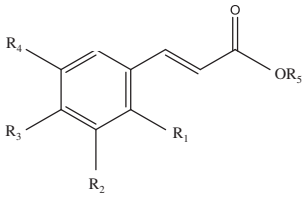
Doubly deionized water with a conductivity of 18.2 MΩ was obtained by using a Milli-Q system (Millipore, Bedford, MA, USA).

All the solutions were filtered with 0.2 μm disposable syringe filters of regenerated cellulose (Symta, Madrid, Spain) before injection into the instrument.

2.2. Avocado samples

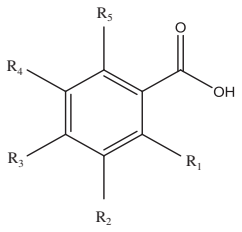
Thirteen different varieties of avocado fruit (Harvest, Sir Prize, Hass, Jiménez 1, ColinV 33, Tacambaro, Lamb Hass, Hass Motril, Pinkerton, Nobel, Jiménez 2, Marvel and Gem) were collected in 2010 at two different ripening degrees; the first one was the fruit just harvested and the second one when the avocado was ready

HYDROXYCINNAMIC ACIDS SKELETON



Compound	R_1	R_2	R_3	R_4	R_5
trans-cinnamic acid	-	-	-	-	-
Chlorogenic acid	-	-	OH	OH	$C_7H_{12}O_6$
Sinapic acid	-	OCH_3	OH	OCH_3	-
m-coumaric acid	-	OH	-	-	-
Ferulic acid	-	OCH_3	OH	-	-
o-coumaric acid	OH	-	-	-	-
p-coumaric acid	-	-	OH	-	-
Caffeic acid	-	OH	OH	-	-

HYDROXYBENZOIC ACIDS SKELETON



Compound	R_1	R_2	R_3	R_4	R_5
Homovanillic acid	-	OCH_3	OH	-	-
Isovanillic acid	-	OH	OCH_3	-	-
Gentisic acid	OH	-	-	OH	-
Benzoic acid	-	-	-	-	-
Syringic acid	-	OCH_3	OH	OCH_3	-
γ-resorcylic acid	OH	-	-	-	OH
α-resorcylic acid	-	OH	-	OH	-
Vanillic acid	-	OCH_3	OH	-	-
o-pyrocatechuic acid	OH	OH	-	-	-
4-hydroxybenzoic acid	-	-	OH	-	-
Gallic acid	-	OH	OH	OH	-
β-resorcylic acid	OH	-	OH	-	-
Protocatechuic acid	-	OH	OH	-	-

Fig. 1. Structures of the phenolic acids included in the standard mix used for the optimization.