



# A simple analytical method for determining inorganic anions and formate in virgin olive oils by capillary electrophoresis with capacitively coupled contactless conductivity detection



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## ABSTRACT

This paper describes a novel and simple method for determining chloride, nitrate, sulfate, fluoride, and formate in virgin olive oil (VOO) by capillary electrophoresis with capacitively coupled contactless conductivity detection. The anions were extracted from VOO by a simple ultrasound-assisted aqueous liquid–liquid extraction procedure. The anions were separated in less than 3 min with good peak resolutions using a background electrolyte composed of 15 mmol L<sup>-1</sup> histidine solution at pH 4.0, adjusted with lactic acid, and 0.6 mmol L<sup>-1</sup> cetyltrimethylammonium hydroxide as electrosmotic flow (EOF) modifier. Tartrate was used as internal standard (I.S.). The proposed method showed good linearity and had coefficients of determination (R<sup>2</sup>) ranging from 0.9984 to 0.9999. The limits of quantification (LOQ) were between 0.01 and 0.7 mg kg<sup>-1</sup>. Recovery tests were used to evaluate the efficiency of the extraction procedure; the recovery percentages ranged from 70.2 to 118%, with a relative standard deviation (RSD) varying from 0.7 to 19.4%. The proposed method was applied to the determination of the anions in five different commercial samples of VOO.

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## 1. Introduction

The consumption of virgin olive oil (VOO) has been increasing annually worldwide, including in Brazil. This increased popularity of VOO is attributed to studies suggesting that people who regularly consume these oils appear to be less prone to heart disease (Cicerale, Lucas, & Keast, 2012; Kesen, Kelebek, & Selli, 2014). Moreover, VOO is an important source of healthful lipids and micronutrients for humans (Garcia-Gonzalez, Aparicio-Ruiz, & Aparicio, 2008). In addition to the interest from olive oil consumers in the health benefits of VOO, these consumers are concerned about the quality and the integrity of this foodstuff, requiring monitoring for adulteration and contamination with toxic species such as heavy metals (Bakkali, Martos, Souhail, & Ballesteros, 2012; Brkljaca, Giljanovic, & Prkic, 2013).

According to the International Olive Oil Council (IOOC) (<http://www.internationaloliveoil.org>), a VOO after its extraction must

only be processed by washing, decantation, centrifugation, and filtration. These treatments should not modify the physical and chemical properties of the oils. However, the presence of inorganic anionic species such as chloride, nitrate, sulfate, and fluoride in VOO is common because these species are naturally present in the raw materials used in VOO production and can be introduced during the industrial manipulation of the oil (Buldini, Ferri, & Sharma, 1997; Clodoveo, Dipalmo, Schiano, La Notte, & Pati, 2014; Dugo et al., 2007).

The inorganic anions in VOO might be beneficial to human health, depending on their concentrations. For instance, fluoride is important for skeletal bone and dental integrity in humans, but in excess it may cause fluorosis (Jackson et al., 2002; Ozsvath, 2008). Chloride is one of the most common anions found in foods and plays an important role in the metabolic acid–base equilibrium. However, chloride promotes serious adverse effects when it is present in high concentrations in humans (Buldini, Cavalli, & Trifiro, 1997). Nitrate is generally present in VOO because of the widespread use of nitrogenous fertilizers. This anion is not very toxic, but it can be reduced to nitrite in the human digestive tract, which has some toxicity due to its conversion into carcinogenic nitrosamines (Jakszyn & Gonzalez, 2006). Sulfate has low toxicity,

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and its presence in VOO can come from the soil or the use of pesticides, such as copper sulfate, in olive cultivation (Buldini, Cavalli, et al., 1997; Buldini, Ferri, et al., 1997).

Small organic anions can also be found in VOO, particularly formate (formic acid), which is produced by oxidation processes (Kiritsakis, Kanavouras, & Kiritsakis, 2002) during the deterioration of the oil. Therefore, measuring the concentration of formate in VOO may be important for quality control purposes.

Because of the different roles that the above mentioned anions play in VOO, their quantification is important in terms of nutritional, toxicological, and technological issues. Currently, the most-used techniques for the determination of small anions in foods are ion chromatography (Buldini, Cavalli, et al., 1997; Buldini, Ferri, et al., 1997; Dugo et al., 2007; Gomez-Ordóñez, Alonso, & Ruperez, 2010; Licata et al., 2013; Lopez-Ruiz, 2000; Silveira, de Caland, & Tubino, 2014) and capillary electrophoresis (CE), particularly using capacitively coupled contactless conductivity detection ( $C^4D$ ) (Kuban & Bocek, 2012; Kuban, Kobrin, & Kaljurand, 2012; Neaga, Iacob, & Bodoki, 2014). Nevertheless, the determination of small anions in VOO by CE has never been reported in literature, and only one work was found reporting this analysis by ion chromatography (Dugo et al., 2007). These findings and the importance of the chemical analysis of VOO suggest that there is a demand for the development of novel, rapid, and simple analytical methods devoted to the determination of inorganic and small organic anions in VOO.

CE- $C^4D$  is a powerful technique employed in the determination of ionic and ionizable analytes, particularly for non-UV-absorbing species with high electrophoretic mobilities. This technique has been used for the determination of several inorganic and organic species in different matrices (Kuban & Hauser, 2009, 2013). In regards to analysis of natural samples, CE- $C^4D$  has been applied for determination of flavonoids in medical plants (Bachmann, Huck, Bakry, & Bonn, 2007), cations in olive oils (Lemos, Pinheiro, Cassella, & Jesus, 2014), ions in coconut waters (Richter, de Jesus, Munoz, do Lago, & Angnes, 2005), and oleic acid in soybean oils (Bockel et al., 2014). In a previous work (Lemos et al., 2014), we demonstrated that CE- $C^4D$  is suitable for the determination of inorganic cations such as  $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$  in VOO, after their liquid–liquid extraction.

This paper is the first to propose a method using CE- $C^4D$  for the determination of  $Cl^-$ ,  $NO_3^-$ ,  $SO_4^{2-}$ ,  $F^-$ , and formate in VOO samples after a simple aqueous liquid–liquid extraction assisted by ultrasound energy. The method was applied to the analysis of five different commercial samples of VOO.

## 2. Material and methods

### 2.1. Samples, reagents, and solutions

All of the reagents were of analytical grade, except methanol, which was HPLC grade from J.T. Baker (Edo de Méx, Mexico). Lactic acid solution 90% (w/w) was obtained from Merck (Darmstadt, Germany), and L-histidine was supplied by Fluka (Tokyo, Japan). The salts NaCl, NaF,  $NaNO_3$ ,  $MgSO_4 \cdot 7H_2O$ , and sodium formate were purchased from Synth (Diadema, Brazil). Tartaric acid and hexadecyl-trimethylammoniumbromide (CTAB) were obtained from Sigma–Aldrich (Steinheim, Germany). Anion exchange resin was acquired from Vetec (São Paulo, Brazil). Ultra-pure water was obtained from a Direct-Q 3 UV Water Purification System (Millipore, Molsheim, France). Cetyltrimethylammonium hydroxide (CTAOH) solution was obtained by passing a  $10 \text{ mmol L}^{-1}$  CTAB solution through an anion exchange resin.

For the CE separations, the background electrolyte (BGE) was composed of  $15 \text{ mmol L}^{-1}$  histidine, adjusted to pH 4.0 with the

lactic acid stock solution. CTAOH was added ( $0.6 \text{ mmol L}^{-1}$ ) to the BGE as an electrosmotic flow (EOF) modifier instead of CTAB to avoid the interference of bromide ions. Standard stock solutions of the anions were prepared by dissolving the respective salts at  $10 \text{ mmol L}^{-1}$  in methanol, except sodium fluoride, which was dissolved in ultra-pure water. The working standard solutions used to obtain the calibration curves were prepared by diluting the stock solutions with a 10-fold diluted BGE solution. The analyzed VOO samples were purchased from local markets in Campinas (Brazil).

### 2.2. CE instrumentation and procedure

The CE separations were conducted in a homemade CE system equipped with an also in-house made  $C^4D$  (da Silva & do Lago, 1998). A wide-bore fused silica capillary column with a 60 cm length (52.0 cm effective) and a  $75 \mu\text{m}$  internal diameter was used in the CE separations. The samples and the standard solutions were hydrodynamically injected into the capillary column by pressure (10 kPa) for 5 s at ambient temperature (20–25 °C). The separation potential was  $-20 \text{ kV}$ , and the  $C^4D$  was operated at 610 kHz (sinusoidal) and 2.0 V (peak to peak amplitude). Before the CE analyses, the capillary column was conditioned by sequential flushes of NaOH solution ( $1 \text{ mol L}^{-1}$ ), water, and BGE, for 5 min each. After each separation run, the capillary was flushed with BGE for 30 s. External calibration method was applied for the quantitative analysis, except for the formate analysis, which required the application of the standard addition method to compensate for the matrix effect. For both methods, the calibration curves were plotted as the ratio of peak area of the analyte to that of internal standard versus analyte concentration. Linear regression was performed on the calibration curves using the least-square method to obtain the coefficients of determination ( $R^2$ ) and the regression equations. The limits of detection (LOD) and quantification (LOQ) were calculated as the concentrations that yielded a signal-to-noise (S/N) ratio of 3 and 10, respectively. The software Origin 8.1 (OriginLab, Northampton, MA, USA) was used for peak integration and statistical analysis.

### 2.3. GC-MS instrumentation and procedure

A gas chromatography–mass spectrometry (GC-MS) analysis was performed to identify the formate ions in the VOO samples. The analysis was conducted using an GC-MS system, model 7890 GC (Agilent Technologies, Santa Clara, CA, USA), equipped with a 7693 autosampler and a 5975C inert XL MSD with a triple-axis detector, operating in selected ion monitoring (SIM) mode. Ionization was performed through the electron impact technique. The chromatographic column was a narrow-bore (30 m length, 0.250 mm i.d., 0.25  $\mu\text{m}$  of film) GC-Agilent HP-FFAP. The oven temperature was held at 40 °C (20 °C/min) for 4 min; afterward, it was increased to 220 °C (30 °C/min) and maintained at this temperature until 13.8 min. The total run time was 14 min, with a solvent delay time of 8.7 min. One microliter of the samples was injected (with a 1:1 sample split) into the GC-MS system. All MS spectra of the samples were compared to the spectra of the formic acid obtained with a standard solution and with the spectrum stored in the equipment's library.

### 2.4. Liquid–liquid extraction assisted by ultrasound

The anions were extracted from VOO as described by Lemos et al. (2014) with a few modifications. For the determination of chloride, nitrate, sulfate, and fluoride, a mass of 10.0 g of the VOO samples was weighted directly in a Falcon tube (15 mL). For the determination of formate, a lower mass (1.0 g) of sample was

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