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Analytical Methods

Characterisation of the phenolic compounds retained in different organic and inorganic filter aids used for filtration of extra virgin olive oil

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ABSTRACT

The aim of this work was to identify and quantify some of the phenolic compounds retained by different filters used for the filtration of two varieties of extra virgin olive oil (Arbequina and Picual). This was performed by HPLC–ESI–TOF and Folin–Ciocalteau spectrophotometric technique. A significant loss in the phenolic concentration with all the tested filter aids was observed. This suggests that the organic filter aids present a higher performance than traditional industrial filters on a laboratory scale, although they show more retentive power regarding the phenolic concentration.

1. Introduction

Extra virgin olive oil (EVOO) presents physiological properties that make it a functional food. From a chemical point of view, olive oil can be divided into major and minor fractions. The major one includes glycerols which represents approximately 98% of the total, also known as the acylglycerol fraction. In the minor fraction, different chemical compounds can be found such as tocopherols, polyphenols, aromatic hydrocarbon compounds, aliphatic and triterpenic alcohols, sterols and volatile compounds. Amongst all of these, the phenolic compounds have a significant importance for their nutritional and technological properties. Their antioxidant function and anti-inflammatory effect (Perona, Cabello-Moruno, & Ruiz-Gutierrez, 2006) have been related to the preventive action on certain diseases such as atherosclerosis (Huang & Sumpio, 2008) and cancer (Giovannini et al., 2008; Menendez et al., 2007). Polyphenols also present an important technological value due to their influence on sensory characteristics (Baccouri et al., 2008; Bendini et al., 2007; Carrasco-Pancorbo et al., 2005) and the shelf life of virgin olive oil.

There are multitude of factors that influence the polyphenolic content of EVOO including agronomic conditions (Servili et al., 2007; Tovar, Motilva, & Romero, 2001), climate, degree of ripening (Bonoli, Bendini, Cerretani, Lercker, & Toschi, 2004), olive variety and production process (Servili, Taticchi, Esposto, Selvaggini, & Montedoro, 2002).

EVOO is exclusively produced through mechanical and physical processes (European Community, Commission Regulation, 2001). The steps of the production process are: collecting, washing, pressing, decantation, centrifugation, storage, filtration and bottling. The effects of pressing, centrifugation and storage on the polyphenolic content in olive oil have been widely studied (Angerosa, Mostallino, Basti, Vito, & Serraiocco, 2000; Morello, Motilva, Tovar, & Romero, 2004; Parenti, Spugnoli, Masella, & Calamai, 2008; Servili et al., 2007). However, there is little information available on the filtration procedure. As a final step in the elaboration process, the filtration may affect the polyphenolic content in olive oil. This process can be carried out with various materials in combination with filtration hardware to improve filtration performance. These materials, denominated filter aids, can be produced from a wide variety of raw materials and their use depends on the final purpose. The olive oil can also be filtered to obtain an impeccable commercial presentation and to remove humidity. In this case cotton or paper filters can be used in the filtration process. Their effect on phenolic fraction has been studied (Gómez-Caravaca et al., 2007; Lozano-Sánchez, Cerretani, Bendini, Segura-Carretero, & Fernández-Gutiérrez, 2010) and it has showed a significant decrease in hydroxytyrosol on EVOO after cotton filtration to remove humidity. However, filtration with either cotton or paper plus anhydrous sodium sulphate led to an apparent increase in the phenolic content.

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Table 1
Filtration times of the different filter aids used in filtration process to laboratory scale.

Filter aids (1 g)	EVOO		Filtration times
	Variety	Quantity (g)	
Earth diatomaceous	Arbequina	70	5 h
	Picual	70	5 h 15 min
Filtracel® 1000	Arbequina	70	3 h 45 min
	Picual	70	4 h
Starch	Arbequina	70	3 h 30 min
	Picual	70	3 h 45 min
Vitacel® L90	Arbequina	70	3 h 30 min
	Picual	70	3 h 45 min

When the olive oil has a high content in suspended solids, the filtration process is carried out using diatomaceous earth. Diatomite is the fossilised remains of microscopic algae. It is mainly formed of silica and it presents great chemical stability. Recently, organic fibrous materials are becoming more and more popular and cellulose fibres and starch are possible candidates. The effect of diatomaceous earth and organic filter aids to measure the content in polyphenols has been not evaluated. This filtration system generates a solid waste that can be an alternative source of polyphenols.

The aim of this study was to evaluate the effect of filter aids on the phenolic content of EVOO. The characterisation of some of the retained phenolic compounds was achieved and the comparison between the results obtained by organic and inorganic filter aids was finally discussed.

2. Materials and methods

2.1. Samples

The unfiltered olive oils used in this study were from two varieties (Arbequina and Picual) obtained from different geographic zones in Andalusia (Spain), the EVOOs were produced in the same year (September 2008) and from the same production plant (*Aceites Maeva* S.L.). Olives were processed by a continuous industrial plant equipped with a hammer crusher, a horizontal malaxator, and a two-phase decanter. Samples were stored in bottles without headspace at room temperature and in darkness before analysis.

2.2. Chemicals and apparatus

All chemicals were of analytical reagent grade and used as received. Methanol and *n*-hexane were purchased from Lab-Scan (Gliwice, Sowinskiego, Poland). Acetic acid and acetonitrile were purchased from Fluka, Sigma–Aldrich (Steinheim, Germany), and Lab-Scan (Gliwice, Sowinskiego, Poland) respectively. Solvents were filtered using a Solvent Filtration Apparatus 58061 (Supelco, Bellefonte, PA, USA). Double-deionised water with conductivity less than 18.2 MΩ was obtained with a Milli-Q system (Millipore, Bedford, MA, USA). Folin–Ciocalteau's phenol reagent and anhydrous sodium carbonate were purchased from Fluka, Sigma–Aldrich (Steinheim, Germany) and Panreac (Barcelona, Spain) respectively. Standards of hydroxytyrosol, tyrosol, vanillin, vanillic acid, *p*-coumaric acid, ferulic acid, luteolin, apigenin, were purchased by Sigma–Aldrich (St. Louis, MO, USA), and (+)-pinoresinol was acquired from Arbo Nova (Turku, Finland). *Celite*®545 and *Kenite*®700, diatomaceous earth used in filtration process, were purchased from Brenntag Quimica (Sevilla, Spain) and World Minerals (Murat, France), respectively. Cellulose fibres (*Filtracel*®1000 and *Vitacel*®L90) and pregelatinised starch from corn were kindly provided by Verbiotech (Granada, Spain).

The vacuum pump used for this work was a Millipore pump model WP6222050 (Millipore, Billerica, MA, USA).

2.3. Filtration process

The filtration methodology consisted in building the filtration column: 1 g of each used filter aid was placed in a 6 mL cartridge (the frits were previously inserted at the bottom of the cartridge). Cellulose fibres (*Filtracel*®1000 and *Vitacel*®L90), pregelatinised starch from corn and diatomaceous earth were used as filter aids. The filtration columns were connected to the vacuum system, and 70 g of EVOO was passed through the cartridge and collected. The relation between the filter aid and the olive oil (1 g/70 g) was selected accordingly to the same scale which is usually applied in the industry. Therefore, the proportions of diatomaceous earths were 0.75 g of *Celite*®545 and 0.25 g *Kenite*®700.

2.4. Extraction procedure of phenolic fraction retained from filter aids

A comparative study of various procedures with different conditions was performed to extract the phenolic compounds from the filter aids. The best results were obtained with this procedure: the cartridge used for filtering was washed with 20 mL of hexane to remove the lipophilic fraction. Then, 40 mL of methanol were passed through the cartridge and collected to remove the hydrophilic fraction. The methanol was finally evaporated under vacuum at 40 °C, and reconstituted in 1 mL of methanol.

2.5. HPLC analysis of phenolic compounds from filter aids

The qualitative analysis was performed using an Instrument Agilent 1200 HPLC system of the Series Rapid Resolution (RRLC). As a stationary phase a Zorbax Eclipse Plus C_{18} analytical column (4.6 × 150 mm, 1.8 μm particle size) was used. Mobile phases A and B were water with 0.5% acetic acid, and ACN, respectively. The chromatographic method consisted of a linear gradient from 5% to 95% B during 23 min, from 23 to 25 min returning to initial conditions in two min, and finally a conditioning cycle of 10 min with the same conditions for the next analysis. The flow rate used was 0.80 mL/min. The temperature of the column was maintained at 25 °C. A volume of 10 μL of the methanolic extracts was injected.

2.6. MS analysis of phenolic compounds from filter aids

MS was performed using the microTOF™ (Bruker Daltonik, Bremen, Germany) which was coupled to the HPLC system and equipped with an ESI interface operating in negative ion mode. Calibration was performed with sodium formate clusters (5 mM sodium hydroxide in water/isopropanol 1/1 (v/v), with 0.2% of formic) in quadratic + high precision calibration (HPC) regression mode. The calibration solution was injected at the beginning of the run and all the spectra were calibrated prior to polyphenol identification. The optimum values of source parameters were: capillary voltage of +4 kV; drying gas temperature, 190; drying gas flow, 8 L/min; nebulising gas pressure, 2 bar and end plate offset, −0.5 kV. The values of transfer parameters were: capillary exit, −120 V; skimmer 1, −40 V; hexapolo 1, −23 V, RF hexapolo, 50 Vpp and skimmer 2, −22.5 V. The source and transfer parameters were established for good sensitivity and reasonable resolution of the mass range for the compounds of interest (50–1000 *m/z*) in order to improved their ionisation performance.

The accurate mass data for the molecular ions were processed using the Data Analysis 3.4 (Bruker Daltonik), software which provided a list of possible elemental formulas by using the Generate Molecular Formula™ Editor. The latter uses a CHNO algorithm providing standard functionalities such as minimum/maximum ele-