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Virgin olive oil *ortho*-phenols—electroanalytical quantification

Teodor Adrian Enache [a,b], Aziz Amine [b], Christopher M.A. Brett [a], Ana Maria Oliveira-Brett [a,*]

[a] Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3004-535 Coimbra, Portugal
[b] Laboratoire Analyses Chimiques et Biocapteurs, Faculté des Sciences et Techniques, Université HassanII-Mohammedia, B.P. 146, 20800 Mohammedia, Morocco

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ABSTRACT

An electroanalytical methodology was developed for the determination of the total *ortho*-phenol content of virgin olive oil (VOO) with high sensitivity and reproducibility. The VOO *ortho*-phenol content depends on its freshness and is normally expressed as HT equivalent. Screen-printed electrodes were used with cyclic voltammetry to investigate the oxidation of catechol, phenol, hydroxytyrosol (HT), tyrosol, caffeic acid and ferulic acid. The oxidation of *ortho*-phenols and *mono*-phenols occurs following different mechanisms, and at different potentials. Using screen-printed electrodes and square wave voltammetry, an HT detection limit of 0.40 μM, was obtained. The electroanalytical methodology developed was applied to the determination of *ortho*-phenol content in fresh and old VOO. The HT equivalent determined for a two-year-old VOO sample was 3 mg/kg, for one-year-old samples was 6–7 mg/kg, and for a fresh VOO sample 30 mg/kg, recoveries in the range of 78–93% of HT standard being obtained. The effect of VOO matrix components on the HT standard response was investigated.

1. Introduction

The beneficial effects of virgin olive oil (VOO) can be attributed not only to the high relationship between unsaturated and saturated fatty acids of olive oil, but also to the antioxidant properties of its phenolic compounds [1,2]. Phenols make up a part of the so-called "polar fraction" of VOO, which is usually obtained by extraction with methanol/water mixtures [2,3]. The olive pulp contains these compounds, which are hydrophilic, but they are also found in the oil [3]. This class of phenols includes numerous components, simple phenolic compounds, such as vanillic, gallic, coumaric and caffeic acids, tyrosol and hydroxytyrosol and more complex compounds such as the secoiridoids oleuropein and ligstroside, and the lignans acetoxypinoresinol and pinoresinol, Scheme 1 [4,5].

VOO is produced using only the cold-pressing method, without further treatment other than washing, filtration, decantation, or centrifugation, and is composed of a triglyceride fraction (up to 90–99% of the olive fruit) and a non-glycerol or unsaponifiable fraction (0.4–5% of the olive fruit) which contains phenolic compounds [2–6]. The phenolic fraction contains at least 36 structurally distinct phenolic compounds, but the widespread phenols of VOO are the secoiridoids, that are characterized by the presence of either elenolic acid or elenolic acid derivatives in their molecular structure [2–5,7]. Breakdown products of two major phenolic constituents of the olive fruit, oleuropein and ligstroside, form the majority of the phenolic fraction [5,7,8]. However, the most abundant secoiridoids of VOO are the dialdehydic form of elenolic acid linked to hydroxytyrosol or tyrosol and an isomer of the oleuropein aglycone [5].

Variations in the phenolic concentration between different VOO are due to numerous factors including: the variety of the olive fruit, the region in which the olive fruit is grown, the agricultural techniques used to cultivate the olive fruit, the maturity of the olive fruit at harvest, and the olive oil extraction, processing, storage methods and time since harvest [9].

Various studies *in vivo* and *in vitro* demonstrated that olive oil phenolic compounds have positive effects on certain physiological parameters, such as plasma lipoproteins, oxidative damage, inflammatory markers, platelet and cellular function, antimicrobial activity and bone health [1–5].

Phenolic compounds can act as antioxidants in various ways. In oxidative systems using transition metals such as Cu and Fe, they can chelate metal ions, which can prevent their involvement in Fenton reactions that can generate high concentrations of hydroxyl radicals [10,11]. However, the most important antioxidant activity is related to the free radical-scavenging ability, by breaking the chain of reactions triggered by free radicals [2,3,7–9].

Numerous studies on the phenols have shown that the degree of antioxidant activity is correlated with the number of hydroxyl groups [9,12–15]. In particular, *ortho*-phenolic substitution, as in hydroxytyrosol, gives high antioxidant ability, while a single hydroxyl substitution, as in tyrosol, does not confer any activity,

* Corresponding author. Tel./fax: +351 239 835295.
E-mail address: brett@ci.uc.pt (A.M. Oliveira-Brett).

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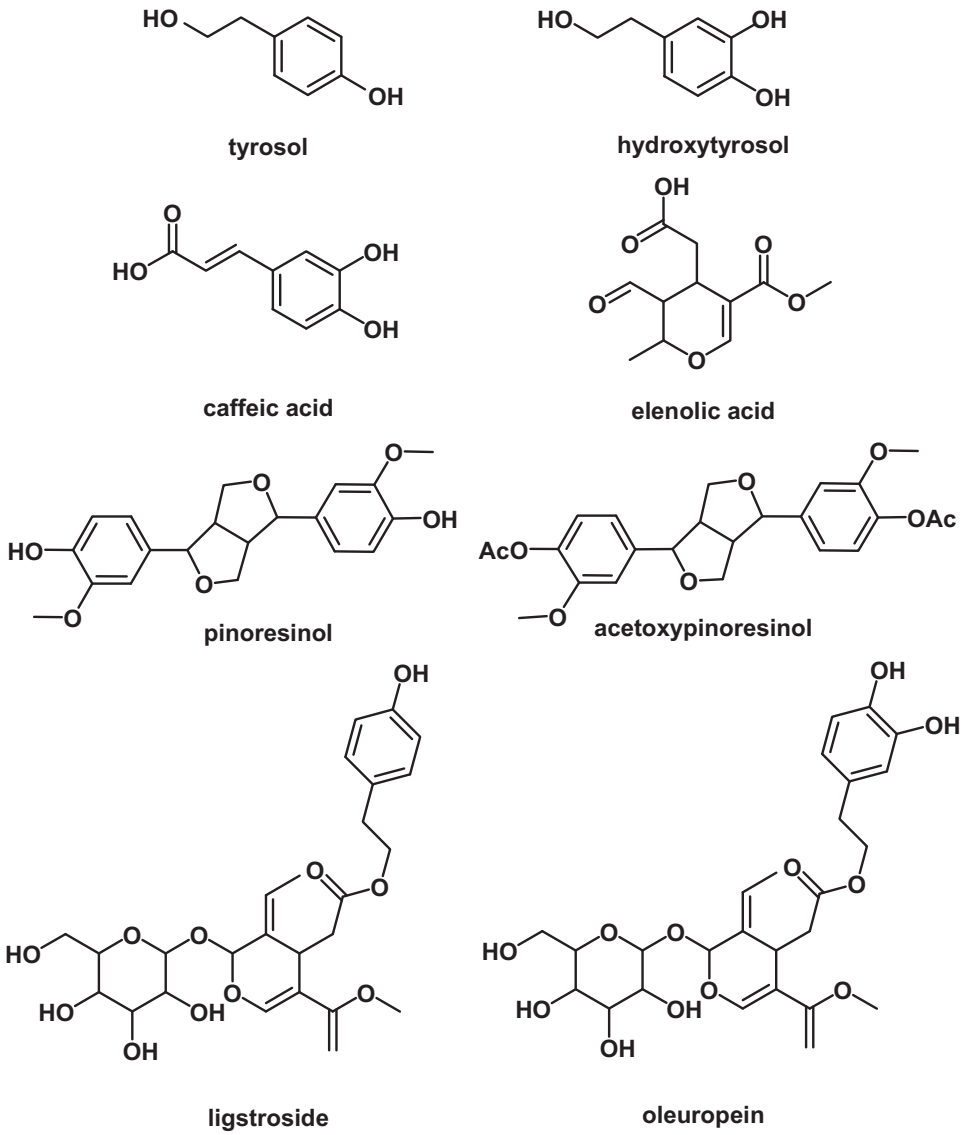

Scheme 1. Chemical structures of phenols present in virgin olive oil (VOO).

since tyrosol does not protect LDL from chemically induced oxidation [8,15].

As a consequence of the antioxidant activity and health benefits there is increasing interest in the determination of the concentration of VOO *ortho*-phenols. The current analytical procedure involves three basic steps: extraction from the oil sample, analytical separation, and quantification [1,5,7,8]. A large number of analytical determinations, based on spectrophotometric methods, after analytical separation, by gas chromatography, high-performance liquid chromatography, and capillary electrophoresis, have already been developed and described [5,7–9,15,16]. However, these methods are expensive, need pre-treatment and require skilled operators.

Voltammetric methods, due to their high sensitivity, have been successfully used for the electrochemical investigation of biological active substituted phenols, providing valuable results [17–19]. The analytical determination of the mostly widespread phenols in aqueous solutions and in complex aqueous food samples, such as teas, wines, beers and others, has been previously investigated using electrochemical techniques at different electrode materials: glassy carbon, carbon paste, boron doped diamond, etc. [17,20–25].

Oxidation of *mono*-phenols is an irreversible process, occurring in one step at a relatively high potential, whereas *ortho*-phenol oxidation occurs at a low potential in a two-electron–proton reversible mechanism [26].

The present study is concerned with the development of a selective electroanalytical method for the determination of VOO total *ortho*-phenol content using square wave voltammetry at screen printed electrodes. This will provide an easy method for VOO total *ortho*-phenol quantification, inexpensive, portable and with the possibility of using a miniaturized device.

2. Experimental

2.1. Materials and reagents

Phenol (Ph), catechol (CT), tyrosol (T), hydroxytyrosol (HT), ferulic acid (FA) and caffeic acid (CA) from Sigma were used without further purification. Stock solutions of 10 mM were prepared in ultra-pure water, ethanol and methanol (1:1:1) and were stored at $+4$ °C.