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Drying at high temperature for a short time maximizes the recovery of olive leaf biophenols

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ABSTRACT

In the current study, recovery of phenolic compounds from fresh, air-dried, freeze-dried and oven-dried (at 60 °C and 105 °C) olive leaves was investigated. The phenol content and antioxidant activity were assessed by gross quantitative methods such as total phenol content (Folin–Ciocalteu's method), total flavonoid content, total *o*-diphenol content and total antioxidant capacity using $ABTS^{+\circ}$ and $DPPH^{\circ}$ scavenging assays. In addition, the phenolic composition of extracts was determined by high performance liquid chromatography (HPLC) equipped with diode array detection (DAD) with tandem mass (MS/MS) and the contribution of individual phenolic components to the antioxidant activity of extracts were evaluted by online ABTS scavenging assay. Extracts obtained from oven-dried leaves at 105 °C showed the highest phenol recoveries and antioxidant activities, whereas extracts obtained from oven-dried leaves at 60 °C had the lowest values. Oven drying of olive leaves at 105 °C for three hours increased oleuropein recovery up to 38 fold as compared with fresh olive leaves. Our results stress the paramount importance of sample pre-treatment in the preparation and analysis of herbal medicines. Futhermore, we highlight the limitations of sole dependence on gross assessment of total phenolic composition and total antioxidant activity in studying plant samples.

1. Introduction

Olive leaf extract (OLE) is gaining popularity in the global nutraceutical market due to a plethora of claimed health attributes. OLE was traditionally consumed for the treatment of a wide spectrum of ailments such as fever, malaria, colic, alopecia, paralysis, rheumatism, gout, sciatica, hypertension, arrhythmia, diabetes and cancer (Flemmig et al., 2011; Waterman and Lockwood, 2007). The functional properties of OLE are essentially due to its biophenol content (Obied et al., 2012). Biophenols are reactive phytochemicals that can undergo a myriad of chemical reactions (Obied et al., 2005b). Olive biophenols can attenuate oxidative stress and prevent oxidative damage through diverse mechanisms, including free radical scavenging, chain reaction breaking, metal chelation and induction of endogenous antioxidant enzymes (Obied et al., 2009, 2012; Servili et al., 2009; Visioli et al., 2002).

The literature shows large qualitative and quantitative variation in the biophenolic composition of OLE (Ahmad-Qasem et al., 2013; Bouaziz and Sayadi, 2005; Hashemi et al., 2010; Hayes et al., 2011; Paiva-Martins and Pinto, 2008). Geography, environmental factors, genetics, harvesting time, agronomic practices, infestation and post-harvest processing can affect the phenolic composition of olives (Goímez-Caravaca et al., 2008; Obied et al., 2008a,b; Vinha et al., 2005). Furthermore, recovery of olive biophenols is influenced by experimental parameters such as extraction technique, solvent, pH, time and temperature (Obied et al., 2005a). Sample handling, processing, clean-up and storage conditions, extract stability, analytical technique sensitivity and the purity of standards used for preparation of calibration curves are commonly overlooked factors that can account for the wide variation in values in the literature. Oleuropein is the major biophenol found in virtually all studied OLE, with rare exceptions (Ryan et al., 2002). Published values for oleuropein recovery from olive leaves varied massively, from 5.6 to 108.6 mg/g dry weight (Ansari et al., 2011; Ranalli et al., 2005; Tayoub et al., 2012). In one study, there was a marked variation, with up to a 10-fold change in oleuropein content based on genetics, season, and the colour/age of the leaves (Ranalli et al., 2005). Furthermore, it is difficult to compare results from different studies, as there is no consensus on how to express the recovery data .i.e. fresh weight, dry weight (DW) or extractable matter (EM).

Analysis of fresh plant materials is considered an ideal situation, to avoid artefacts resulting from sample degradation or extensive

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clean-up procedures. As it is not always possible and sometimes impossible to analyse fresh plant samples, several drying techniques have been proposed. Drying has been always considered a sub-optimal procedure to preserve plant materials and hence subtle drying techniques are always recommended, such as freeze-drying (Waterman and Mole, 1994). Cheaper alternatives such as air-drying or oven-drying at low temperatures (40–60 °C) have been typically recommended when cost is an issue. Nonetheless, these conventional hypotheses and practices have been challenged by sporadic experimental reports (Ahmad-Qasem et al., 2013; Vinson et al., 2005).

As a drug of botanical origin, olive leaves are pharmacopoeially prepared by air-drying. At the same time, some commercial neutraceutical products proclaim superiority based on the extraction of fresh olive leaves. Currently, there are no widely accepted guidelines for the drying of olive leaves. Data from the literature are insufficient or contradictory. The impact of drying on the nutritive value of olive leaves as animal feed has been investigated (Martín-García and Molina-Alcaide, 2008). Though no attention was given to the phenolic content and antioxidant activity, air-drying was recommended as a simple and cheap technique that preserved the nutritive value of olive leaves. Various drying techniques, such as air-drying, freeze-drying, and oven-drying at low and high temperatures have been used to compare the effect of drying method on biophenol content (Ahmad-Qasem et al., 2013; Hung and Duy, 2012; Julkunen-Tiitto and Sorsa, 2001; Keinänen and Julkunen-Tiitto, 1996). Malik and Bradford reported air drying at ambient temperature (25 °C) as the most suitable method for processing olive leaves for commercial purposes due to its convenience, economic viability and good recoveries of biophenols, particularly oleuropein and verbascoside (Malik and Bradford, 2008). On the contrary, a more recent study identified drying at 120 °C as the best preserving method for recovery of olive leaf biophenols, particularly oleuropein (Ahmad-Qasem et al., 2013). Infra-red drying of olive leaves seemed promising with a three-fold increase in total phenol recovery at 70 °C, yet the impact of drying on individual biophenols was not investigated (Boudhrioua et al., 2009). Therefore, we systematically investigated the impact of different drying conditions on the recovery of different biophenols from olive leaves, so as to resolve the current controversy and provide a basis for optimizing commercial production of OLE.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals and reagents were used without further purification: Folin-Ciocalteu reagent, 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH•), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2′ azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), formic acid, aluminium chloride, sodium nitrate (Sigma–Aldrich, Sydney, Australia), anhydrous sodium sulphate, sodium hydroxide, sodium carbonate (Univar, Sydney, Australia); HPLC-grade methanol (Fisher Scientific. New Jersey, USA); *n*-hexane, glacial acetic acid, ethanol, hydrochloric acid, ammonium acetate (Merck, Melbourne, Australia), ethyl acetate and anhydrous acetonitrile, (UNICHROME, Sydney, Australia).

Gallic acid, chlorogenic acid, caffeic acid, rutin, quercetin, 4-hydroxymandelic acid, 3,4-dihydroxymandelic acid, luteolin, luteolin-7-*O*-glucoside, catechin hydrate, 4-hydroxyphenylacetic acid, syringic acid, 3,4-dihydroxyphenyl acetic acid, homovanillyl alcohol, 4- hydroxybenzoic acid, trans-cinnamic acid, catechin, ferulic acid, *o*-coumaric acid, homovanillic acid, *p*-coumaric acid, sinapic acid, hesperetin, neohesperidin and tyrosol were purchased from Sigma–Aldrich (Sydney, Australia). Apigenin, apigenin-7-*O*-glucoside, diosmin, oleuropein and verbascoside were purchased from Extrasynthese (Genay, France). Hydroxytyrosol was bought from Cayman Chemical Company (Ann Arbor, MI).

Standards were dissolved in 80% aqueous methanol to prepare stock solutions of 1 mg/mL. Quercetin and luteolin had to be dissolved in absolute methanol and rutin was dissolved in warm 80% aqueous methanol, as described previously (Obied et al., 2005b). Water used in all analytical work was purified by a Modulab Analytical water system (Continental Water Systems Corp., Melbourne, Australia).

2.2. Collection of olive leaves

Olive leaves were collected from the Charles Sturt University Olive Grove at Wagga Wagga, NSW, Australia. The mature green leaves were handpicked at the operator height around the whole perimeter of three trees of the same cultivar, from summer through to autumn. Leaves of the Frantoio cultivar were collected in February, 2012. Leaves of the Leccino cultivar were collected in March, 2012 while leaves of Frantoio and Leccino cultivars were again collected in April, 2012, in addition to two other cultivars; Hardies Mammoth and Arbequina. Leaves were collected in plastic bags and brought to the laboratory without any delay. Fresh leaves were analysed or subjected to various drying conditions within 1 h of collection.

2.3. Sample treatments

A pilot study was conducted for optimizing drying time and moisture content for different treatments. In oven-drying at 105 °C, measurements were recorded on an hourly basis for 5 h. For air-drying, leaves were dried at room temperature for ten days and the weight was recorded daily. Weight was recorded after 18 and 24 h for the freeze drying treatment, while measurements were taken every 6 h for 48 h for oven drying at 60 °C. Optimum drying time is the minimum time required to achieve the lowest moisture content with minimal degradation. Our data showed that optimum drying time was 24 h for freeze drying; 48 h for air drying; 6 h for oven drying at 60 °C; and 3 h for oven drying at 105 °C (data not shown).

One portion of fresh leaves was ground in a coffee grinder and extracted to determine the biophenol composition of fresh samples within one hour after collection. Equal aliquots of fresh leaves were immediately subjected to one of the following drying treatments. Air drying: leaves were air-dried at room temperature (21 ± 2 °C) for 48 h. Oven drying at 60 °C: leaves were dried in a forced-air oven at 60 °C for 6 h. Oven drying at 105 °C: leaves were dried in a forced-air oven at 105 °C for 3 h. Freeze drying: leaves were freeze dried in a Christ-Alpha 2–4 LD plus freeze dryer (Biotech International, Germany) for 24 h. Dried leaves were ground in a coffee grinder and stored at −20 °C in air-tight containers until extracted.

2.4. Moisture content

Moisture content was determined using the procedures described by the United States Pharmacopeia (2000) using the gravimetric method for “articles of botanical origin” as described previously (Obied et al., 2005b). Five grams of fresh leaves were dried in an evaporating dish in a convection oven at 105 °C. The leaves were weighed after every hour until the difference between two successive readings was less than 0.25%.

2.5. Extraction of biophenols

Biophenols were extracted according to the method described earlier (Obied et al., 2005a), with slight modification. Two grams of fresh/dried leaf powder was extracted with 10 mL of 80%