Acta Histochemica xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.com/locate/acthis

Olive oil polyphenols extracts inhibit inflammatory markers in J774A.1 murine macrophages and scavenge free radicals

Marwa Abdallah[a,b,*], Stefania Marzocco[c], Simona Adesso[c], Mokhtar Zarrouk[b], Mokhtar Guerfel[b]

[a] University of Tunis El Manar, Faculty of sciences of Tunis, Campus University, Tunis 1060, Tunisia
[b] Laboratory of biotechnology of olive, Center of biotechnology of Borj Cedria, BP 901, 2050, Hammam-Lif, Tunisia
[c] Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, I-84084 Fisciano, Salerno, Italy

ARTICLE INFO

Keywords:
Anti-inflammatory
Antiradical
Tunisian olive oil
Maturity indices
Polyphenols extracts

ABSTRACT

Here we evaluate the olive oil antiradical and anti-inflammatory potential through its polyphenols extracts and examine the influence of olive maturity on olive oil quality properties, polyphenols composition and biological potentials. Samples have been obtained from minor Tunisian olive cultivars (Chemchali, Fouji and Zarrazi) at different maturity indices. Principal quality properties were evaluated and polyphenols analysis was carried out by Folin Ciocalteu reagent and HPLC-UV-MS. Antiradical activity was examined by DPPH and FRAP scavenging assays while J774A.1 murine macrophages were used to evaluate anti-inflammatory potential by analyzing NO production with Griess reagent method and iNOS and COX-2 expression by cytofluorimetric analysis. Our results revealed that quality characteristics, total phenol content, as well as phenolic compound concentrations were significantly affected by the olive maturity levels. On the other hand, the polyphenols extracts showed an interesting radical scavenging capacity and a potential ability to inhibit inflammatory markers at 90% for NO release and 75% for iNOS expression. Thus, our study establishes that olive oil through its polyphenols extracts has a substantial antiradical and anti-inflammatory potential. Likewise a lot of attention should be attributed to olive ripening level in order to decide the optimum harvesting time.

1. Introduction

Over years, the growing popularity of Mediterranean diet has become widely associated with improved health and well-being. It seems to be ascribed to the high intake of olive oil, which represents the main lipid source of this diet. Its healthy properties concern the ability to prevent diseases that may be related to oxidative damages such as inflammations, coronary heart diseases and several types of cancers (Pérez-Jiménez et al., 2007). In addition to the excellent balance between saturated and unsaturated fatty acids, an increasing number of studies have attributed the beneficial effect of olive oil to its minor compounds, particularly phenolic components, which have shown a broad spectrum of bioactive properties including the beneficial effect on plasma lipoproteins, lipid oxidation and platelet function (Cicerale et al., 2010). Polyphenols exhibit also a potential effect on inflammation process (Aparicio-soto et al., 2014; Rosignoli et al., 2013) which can be a harmful phenomenon involved in various diseases such as cancers and high blood pressure (Bautista, 2003; Coussens and Werb, 2002). Macrophages present the major inflammatory effector cells playing a crucial role by involving the initiation of inflammatory responses. These cells are activated by exposure to interferon-ɣ, pro-inflammatory cytokines, and bacterial LPS (Zhang and Ghosh, 2000) which disturb the balance of the intracellular state and leads to an excess of inflammatory mediator production such as ROS and NO in addition to some enzymes like iNOS and COX-2. Olive oil polyphenols are very heterogeneous with at least 36 structurally distinct phenols (Cicerale et al., 2010) belong to different classes based on their molecular weights and structures: phenolic acids, phenyl ethyl alcohols, flavonoids, lignans and secoiridoids (Jolayemi et al., 2016). Moreover, these compounds are important not only for nutritional benefits, but also for sensory quality and shelf-life of olive oil (Condelli et al., 2015). Although it is the only vegetable oil that can be consumed directly in its raw state, olive oil quality is influenced by a great number of factors such as geographical location (Mailer et al., 2010) and agronomic practices (Krichene et al., 2010). It has been suggested that the cultivar and the olive maturity stage are two of the most important ones

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, Ferric Reducing Antioxidant Power; NO, nitric oxide; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; ROS, reactive oxygen species; LPS, lipopolysaccharide; BHT, butylated hyroxytoluene; DMEM, Dulbecco's Modified Eagle's Medium; FCS, foetal calf serum; MTT, 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl- 2H-tetrazolium bromide; TXB, thromboxan; PGS, prostaglandins

* Corresponding author at: Laboratory of biotechnology of olive, Center of biotechnology of Borj Cedria, BP 901, 2050, Hammam-Lif, Tunisia.
E-mail address: marwa.abdallah88@yahoo.fr (M. Abdallah).

https://doi.org/10.1016/j.acthis.2017.10.005
Received 6 July 2017; Received in revised form 16 October 2017; Accepted 16 October 2017

(Gómez-Rico et al., 2008; Matos et al., 2007; Skevin et al., 2003). When olive maturation proceeds, a number of changes occur in the fruit and several metabolic processes take place which involve subsequent variations in the profile of many compounds in olive oil. These changes are reflected on the sensorial characteristics, especially in the aroma, the oxidative stability and/or nutritional value of the obtained olive oil. According to our knowledge, there are few detailed studies on quality characteristics and polyphenols composition for the selected varieties, but no investigations about the antiradical and anti-inflammatory potential in J774A.1 macrophages has been published. Therefore, the aim of this paper was to evaluate the Tunisian olive oil antiradical, and anti-inflammatory potential through its polyphenols extracts and examine the influence of maturity indices on olive oil quality properties, polyphenols composition and biological potentials.

2. Materials and methods

2.1. Olive samples

In this study, olive fruits used for oil extraction were harvested from three minor Tunisian olive cultivars (Chemchali, Fouji and Zarrazi), localized in the oasis of Gafsa in the south of Tunisia. For each cultivar, olives were harvested at different maturity indices.

2.2. Olive oil extraction

The classification of harvested olives according to the different maturity indices was established depending on the international olive council method (International Olive Council, 2011). For our study, three maturity levels were chosen (3, 4, and 5). Olive oil extraction steps has been previously well described (Abdallah et al., 2016).

2.3. Analytical methods

2.3.1. Quality parameters

The determination of free acidity, peroxide value and the specific UV absorbance conventionally indicated by K_{232} and K_{270} were carried out according to the European official methods of analysis (EEC, 1991). Free acidity was expressed as “g” of oleic acid per 100 g of olive oil, peroxide value was given as milliequivalent active oxygen per kg of olive oil (meq O_2/kg), while K_{232} and K_{270} extinction coefficient were obtained from the absorbance at 232 nm and 270 nm respectively.

2.3.2. Oxidative stability

The oxidative stability was evaluated using Rancimat 743 apparatus (Metrohm. Co., Basel, Switzerland). A sample of 3 g of olive oil was heated at 100C° and the air was bubbled through it at a flow rate of 10L/h. The stability of olive oil was expressed as the oxidation induction time (hours) (Gutiérrez, 1989).

2.3.3. Total phenol content

Total phenol content was colorimetrically quantified by Folin-Ciocalteu method (Gutfinger, 1981). Using 2.5 g of olive oil, the polyphenols extract was isolated by adding 5 ml of hexane and 5 ml of methanol/water (60:40 v/v). The methanolic phase containing phenolic compounds was recovered, evaporated under vacuum and stored at −20 °C for subsequent analysis. The total concentration of phenols was estimated by adding 0.5 ml of Folin-Ciocalteu reagent, 5 ml of distilled water and 1 ml of Na_2CO_3 aqueous solution (35% w/v) to 0.2 ml of methanolic fraction. The volume was brought up to 10 ml with distilled water. The mixture was allowed to stand in the dark for 1 h. Absorbance at 725 nm was measured using an UV–vis spectrophotometer. The phenolic fraction was expressed as mg Gallic Acid Equivalent (GAE) per kg of olive oil (mg GAE/kg).

2.3.4. Determination of phenol compounds with HPLC-UV-MS

Chromatographic determination of phenolic compounds was provided by a high performance liquid chromatography (Agilent Technologies, Palo Alto, CA) equipped with a UV–vis detector coupled to an ESI source of a mass spectrometer (MS) (Agilent) where separation was performed using a Zorbax C18 column (4.6 × 150 mm, 5 µm). According to the method described by (García-Villalba et al., 2010), the mobile phases were H_2O (0.5% acetic acid) (Phase A) and acetonitrile (ACN) (Phase B). With a flow rate of 1.5 ml/min, 20 µl of phenol extract was injected and the solvent gradient elution changed as follows: 0–10 min: 5–30% B; 10–12 min: 30–33% B; 12–17 min: 33–38% B; 17–20 min: 38–50% B; 20–23 min: 50–95% B. Finally, the volume of phase B was reduced to the initial state (5%) and the column was re-equilibrated for 10 min. UV–vis detection was set at 200, 240, 280 and 330 nm. The flow rate used for the HPLC (1.5 ml/min) was too high to achieve stable ionization with ESI (maximum flow rate is about 1 ml/min), so it was necessary to use a flow divisor 1:2. In this way, the flow delivered in mass spectrometry was low enough to avoid introducing of moisture in the system. The MS working conditions were: pressure of the nebulizing gas: 7 psi; dry gas flow rate: 6 l/min; dry gas temperature: 190 °C. The spectrum was acquired in the range of 50–800 m/z on the negative mode. Identification of individual phenols was achieved by comparing their retention time with pure standards and their mass spectra. The results were expressed as mg of tyrosol per kg of oil according to the IOC method (International Olive Council, 2009).

2.4. Radical scavenging assays

2.4.1. DPPH assay

The ability of olive oil polyphenols extracts to scavenge DPPH free radical was evaluated following the protocol described by (Brand-Williams et al., 1995) with some modifications. To 100 µl of increasing concentrations of polyphenols extracts (0.25, 0.5, 1 mg/ml) or methanol as control, 3.9 ml of methanolic solution of DPPH (6×10^{-5} M) was added. After keeping the mixtures in darkness for 1 h, the optical density has been measured at 515 nm and the antiradical action toward DPPH radical was calculated. Plotting the values of the percentage of inhibition of DPPH radicals against the different concentrations of phenolic fraction allows a straight line from which the inhibitory concentration EC_{50} (efficient concentration of polyphenols extract necessary to reduce 50% of the DPPH free radicals) was calculated and BHT was used as positive control.

2.4.2. FRAP assay

The FRAP radical scavenging activity has been evaluated according to the method reported by (Oyaizu, 1986) with some modifications. 200 µl of polyphenols extracts at concentrations ranged from 0.15 to 0.5 mg/ml were mixed with 0.5 ml of 200 mmol/l sodium phosphate buffer at pH 6.6 and 0.5 ml of potassium ferricyanide ($K_3F_e(CN)_6$) (1%). After incubation during 20 min at 50 °C, 0.5 ml of tricloroacitic acid (10%) was added and the mixture was centrifuged. The supernatant was recovered and mixed with 0.5 ml of distilled water and 100 µl of 0.1% ferric chloride (F_eCl_3). Concentration providing 0.5 of absorbance (EC_{50}) was calculated from the absorbance graph plotted at 700 nm against the concentration and using ascorbic acid as a positive control.

2.5. Anti-inflammatory activity

2.5.1. Cell culture

J774A.1 murine monocyte macrophage cell line obtained from the American Type Culture Collection (Rockville, MD) was cultured adherent to petri dishes with DMEM enriched with 10% FCS, 25 mM Hepes, 2 mM glutamine, 100U/ml penicillin and 100 µg/ml streptomycin then maintained at 37 °C under 5% CO_2 humidified air. Unless stated otherwise, all reagents and compounds were purchased from Sigma Chemicals Company (Sigma, Milan, Italy).