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Antioxidant effects of extra virgin olive oil enriched by myrtle phenolic extracts on iron-mediated lipid peroxidation under intestinal conditions model

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ABSTRACT

Chelating and free radicals scavenging activities of extra virgin olive oil (EVOO) enriched by *Myrtus communis* phenolic compounds (McPCs), α-tocopherol and Butylated hydroxytoluene (BHT) were evaluated using chemical assays, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Oxygen radical absorbance capacity (ORAC), and biological model as 2,2′-azobis (2-aminopropane) dihydrochloride (AAPH) or Fe^{+3}/Ascorbic acid (Fe^{+3}/AsA) system mediated peroxidation of L-α-phosphatidylcholine aqueous dispersions stabilized by bile salts (BS) under simulated intestinal conditions (pH 7.4). McPC-EEVOO increased significantly the neutralization of DPPH radical and AAPH-derived radicals in ORAC assay more than α-tocopherol and BHT. The phospholipid stability increased by a factor of 33.6%, 34.8%, 19.3% and 10.7% for myrtle microwave assisted extraction (MAE) and conventional extraction (CE) extracts, α-tocopherol and BHT, respectively, as compared to the control (EVOO without enrichment) in Fe^{+3}/AsA system. But a slightly additive effect was observed when AAPH system was used. Our observation showed that McPCs may interact positively with EVOO to inhibit phospholipid peroxidation, and thus, McPC-EEVOO could be a potential functional food.

1. Introduction

Lipid oxidation has been shown to be associated with the disturbance of the structure and the function loss of the cell membranes and the oxidative modification of low-density lipoproteins and is an important initial event for the pathogenesis of atherosclerosis (Marinova, Toneva, & Yanishlieva, 2008). Data related to the oxidative fate of lipids during digestion remain relatively scarce. The acidic gastric environment, combined with the presence of oxygen in high quantities, can favor oxidative reactions. In the upper intestinal compartment, oxygen remains present and the lipid emulsification or micellization induced by the biliary salts could favor the pro-oxidant activity of iron ions ingested concomitantly with polyunsaturated fatty acid (PUFA)-carrying lipids (Kenmogne-Domguia, Moisan, Viau, Genot, & Meynier, 2014). So, lipid oxidation of fatty acids could take place in the digestive tract before their intestinal absorption. Indeed, it is important to take into account that lipid oxidation products could be absorbed by intestinal cells (Kenmogne-Domguia et al., 2014) which could lead to an increase in oxidative stress in the plasma after their absorption. Thus, the inhibition of lipid peroxidation during ingestion is an important role of radical-scavenging dietary antioxidants. In the few studies investigating lipid oxidation in simulated gastric conditions of oil-in-water model emulsions, an effect of quercetin and α-tocopherol on inhibition of heme-induced peroxidation was observed (Goupy, Vulcain, Caris-Veyrat, & Dangles, 2007;

Abbreviations: McPC, *Myrtus communis* phenolic compound; EVOO, extra virgin olive oil; EEVOO, enriched extra virgin olive oil; MAE, microwave assisted extraction; CE, conventional extraction; EYPC, egg yolk phosphatidylcholine; AUC, area under curve; *M. com*, *Myrtus communis*.

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Lorrain, Dangles, Genot, & Dufour, 2009). A recent study (Kenmogne-Domguia et al., 2014) showed also that during *in vitro* digestion, emulsified lipids were oxidized, and endogenous tocopherols were consumed with a formation of a high amount of malonaldehyde at the end of the digestion. Many medicinal plants contain large amounts of antioxidants such as polyphenols, which possess significant antioxidant capacities associated with lower occurrence and lower mortality rates of several human diseases (Aidi Wannes et al., 2010). For this, contemporary dietary programs often recommend certain aromatic and medicinal plants as functional foods. The enrichment of lipid food by natural antioxidant was recently developed as a new way of phytochemical potential application. The enrichment of extra virgin olive oil (EVOO) by green tea phenolic compounds improved significantly the antiatherogenic properties of olive oil resulting in significant attenuation of atherosclerosis development (Rosenblat, Volkova, Coleman, Almagor, & Aviram, 2008). Moreover, in a mixture of phenolic compounds, synergistic or antagonistic effects may occur and this could modulate the total antioxidant activity of the whole food. Subsequently, it is very important to take into account all these interactions when designing a functional food (Palafox-Carlos et al., 2012). According to our knowledge, there is none work about the effect of plant-enriched extra virgin olive oil on peroxidation of a phospholipid aqueous dispersion model under simulated gastrointestinal conditions.

The aim of this work is to evaluate for the first time the free radical scavenging effect and/or chelating ability of extra virgin olive oil enriched by myrtle extracts (obtained by both methods: microwave assisted extraction MAE and conventional extraction CE) on egg yolk phosphatidylcholine/bile salts (EYPC/BS) aqueous dispersion oxidation under simulated intestinal conditions (pH 7.4). The oxidation model was used to simulate the intestinal conditions (pH 7.4) of lipid peroxidation may occurring in small intestine during lipid digestion. For this, we have used EYPC as lipid substrate and bile salts as physiological detergent substances to stabilize the phospholipid aqueous dispersion formed by sonication. AAPH or Fe^{+3}/ascorbic acid system were used to initialize the phospholipid peroxidation. The cryo electronic microscopy was applied to visualize structure and size of formed lipid in the system used (data not shown).

2. Materials and methods

2.1. Chemicals and standards

All chemicals used were of analytical grade. Egg yolkphosphatidylcholine (EYPC), butylatedhydroxytoluene (BHT), and 2 N Folin-Ciocalteu reagent were purchased from Sigma Aldrich Chemical Co. (Saint Quentin Fallavier, France). Gallic acid and 2′,7′-dichloro-fluorescein were obtained from Merck (Darmstadt, Germany). Methanol used for chromatography was HPLC-grade supplied by Merck. Ethanol (EtOH) used for preparing standard solutions was from Prolabo (Paris, France). Chloroform was from Prolabo. 2.2′-Azobis-2-amidinopropane hydrochloride (AAPH) was from Biovalley (Conches, France). Ascorbic acid was purchased from Merck, France. Iron (III) chloride ($FeCl_3$, $6H_2O$) from Labosi-Fischer Scientific, France. Myricitrin was purchased from Roth Sochiel EURL (Lauterbourg, France).

2.2. Plant material preparation

M. communis leaves were collected in the region of Bejaia (Algeria), and dried in an oven at 40 °C until constant weight, then crushed and sieved to have a size less than 125 μm. The samples were stored in the dark at room temperature.

2.3. Extraction procedure

2.3.1. Microwave assisted extraction (MAE) method

The extraction procedure was previously described in our previous study (Dairi et al., 2014). Briefly, a domestic microwave oven (NN-S674MF, LG, Japan, 32 L, 1000 W; variable in 100 W increments, 2.45 GHz) was used to extract myrtle leaf phenolic compounds. The leaf suspension (1 g of leaf powder per 20 mL of EtOH/water, 50/50; v/v) was irradiated by microwaves (700 W of power) for 1 min. After MAE treatment, the sample was filtered and two other additional extractions were carried out. The final extract was stored at 4 °C until further use.

2.3.2. Conventional extraction (CE) method

Samples were extracted with EtOH/water (50/50; v/v) (Dairi et al., 2014). Briefly, 1 g of myrtle powder was mixed with 20 mL of hydroalcohol solvent and blended for 60 min with magnetic agitation at room temperature. After that, the process was the same as for MAE procedure.

2.4. Identification and quantification of McPCs by RP-HPLC

The chromatographic studies were performed as described in our previous report (Dairi et al., 2015) using an Agilent Model 1100 LC instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with UV–Vis diode-array detector and rapid scan fluorescence spectrophotometer detector. The analytical data were evaluated using Chemstation software package. The analytical column employed was a 120 ODS 5 μm, 150 × 4.6 mm (Teknokroma, Barcelona, Spain). The column temperature was set at 25 °C. The mobile phase consisted of water with 0.5% acetic acid and 0.1% acetonitrile (A) and acetonitrile with 0.5% acetic acid (B). The gradient program was as follows: 0–20 min, 10% B; 20–30 min, 45% B; 30–45 min, 45% B; 45–50 min, 10% B; 50–65 min, 10% B. The flow rate was set constant at 0.5 mL min^{-1} and the injection volume was 10 μL. The identification of each compound was based on a combination of retention time and spectral matching.

2.5. Measurement of antioxidant activities

2.5.1. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH·) scavenging assay

The antioxidant activity of sample extracts was measured by bleaching of the purple-colored solution of DPPH· under reduction by an antioxidant compound. The DPPH· solution (60 μmol L^{-1}) was prepared in methanol, and 3 mL of this solution was mixed with 100 μL of samples extracts or of standard BHA or α-tocopherol at various concentrations. The samples were incubated for 20 min at 37 °C in a water bath, and then the absorbance decrease was measured at 515 nm (Dudonne, Vitrac, Coutiere, Woillez, & Merillon, 2009).

The percentage of loss of DPPH· absorbance was calculated according to the following equation: % loss of DPPH· = [(AC (DPPH.) − AA (sample)/AC (DPPH.)] × 100, where AC (DPPH·) is the control absorbance at time = 0 min; and AA (sample) is the absorbance of DPPH· in the presence of antioxidant at time = 20 - min. The antioxidant activity of each test sample and standard is expressed in terms of concentration required to reduce DPPH· absorbance by half (IC_{50} expressed as mg L^{-1}) and calculated from the log-dose inhibition curve.

2.5.2. Oxygen radical absorbance capacity (ORAC)

ORAC values were measured with fluorescence spectrometer (Victor^{2}Wallac–Perkin-Elmer) by inhibition of 2′, 7′dichlorofluorescein (DCF) consumption (Ishimoto et al., 2011) with slight modifications. Briefly, all samples and reagents were dissolved in 10 mmol phosphate L^{-1}/150 mmol NaCl L^{-1} buffers (PBS) at pH 7.4. 50 μL