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Extraction of antioxidant compounds from different varieties of *Mangifera indica* leaves using green technologies

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ABSTRACT

Supercritical Fluid Extraction (SFE) and Subcritical Water Extraction (SWE) from mango leaves were applied in order to obtain extracts with high phenolic content and potent antioxidant activity. The effects of extraction conditions on sub- and supercritical CO_2 extraction were analyzed: temperature (35 and 55 °C), pressure (10 and 40 MPa), percentage of co-solvent (0 and 20%) and type of co-solvent (methanol/ethanol). The best condition (CO_2 + 20% of ethanol at 10 MPa, 55 °C, 20 g/min and 3 h) was compared with SWE (4 MPa, 100 °C, 10 g/min, and 3 h) using seven mango cultivars. SWE was more efficient than subcritical CO_2 + ethanol. The antioxidant activity was evaluated by DPPH assay, and the quantification of the main polyphenols of mango leaves by HPLC analysis. SWE showed global yields up to 35% for Kent variety, and extracts with antioxidant activities superior to (+)-α-tocopherol related with their high content on the polyphenols mangiferin and quercetin.

1. Introduction

The challenges of this century based on a sustainable and more friendly environment development have turned the vision of chemical production toward a new industry concept of biomass refining in order to decrease rapid consumption of non-renewable resources (petroleum, natural gas, coal, and minerals). In the beginning, a typical biorefinery convert essentially natural renewable matter into bio-energy products. However, in the next generation biorefinery, the feedstock will be fractionated further into valuable components by extraction, fermentation and controlled pyrolysis, as well as by more traditional methods.

One of the first stages in the new biorefinery is the extraction of secondary metabolites from low value biomass considering that they are of greater value in cosmetic, nutraceutical and pharmaceutical industries. The use of harmless extraction methods is essential to comply with and environmental compatible and sustainable chemical production [1–3].

Supercritical Fluid Extraction (SFE) and Subcritical Water Extraction (SWE) are interesting alternatives so present several advantages including the use of green solvents, faster and more selective processes, and the low degradation of chemical compounds [4–8]. Both techniques have been widely explored in recent years in order to recover bioactive compounds from diverse plants and agri-industrial by-products [4–22].

Agricultural by-products of mango, particularly leaves and bark, present a high content on potent phenolic compounds, mainly mangiferin and quercetin, whose pharmaceuticals and nutraceutics properties have been demonstrated in several studies [23–30]. Mango is one of the most important tropical fruit worldwide with a global production superior to 38 million tones and an area harvested superior to 5 million hectares in 2010 [31]. Annually pruning activity generates considerable quantities of residues which are usually burned or used for soil amelioration. Thus, conversion of pruning mango residues into valuable chemical products by efficient and low impact extraction techniques results clearly attractive within the concept of biorefinery.

The extraction from mango by-products using SC-CO_2 or subcritical water has not been widely studied. Traditional solvent extraction techniques are still usually used to recover bioactive compounds from mango [23,25–30] despite the drawbacks present in these techniques [8,11–13,16,18].

Mango leaves extracts with antioxidant activity have been obtained by SC-CO_2 extraction [32], but pure CO_2, a nonpolar solvent, provide a low efficiency to extract highly or slightly polar compounds. Thus, the addition of CO_2 modifiers such as alcohol cosolvents should increase the extraction of polar polyphenols and also improve the antioxidant activity of extracts, as described by

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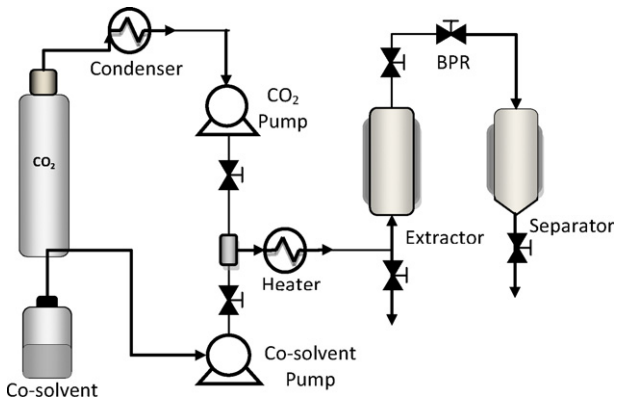

Fig. 1. Schematic diagram of the high pressure equipment.

other authors using different natural matters [7,8,10–15,18]. On the other hand, although SWE is efficient to extract polar compounds, this technique has not been evaluated before using this raw material.

Therefore, in this work Sub- and Supercritical Fluid Extraction from mango leaves using pure CO_2 and CO_2 plus co-solvents at different conditions was studied and compared with SWE. Extracts were evaluated considering the global extraction yield, the antioxidant activity and the phenolic composition. In addition, the performance of seven varieties of mango leaves was analyzed using CO_2 plus co-solvents and SWE.

2. Materials and methods

2.1. Materials

The seven varieties of *Mangifera indica* L. leaves studied (Kensington, Kent, Keitt, Tommy Atkins, Osteen, Ataulfo and Langra) were provided by "Estación Experimental La Mayora", Superior Centre of Scientific Research (CSIC), Málaga, Spain. The leaves were collected in June 2010 and February 2011. All leaves were dried at room temperature until constant weight and kept frozen in the absence of light.

Carbon dioxide (99.995%) was provided by Abello-Linde S.A. (Barcelona, Spain). 2,2-Diphenyl-1-picrylhydrazyl, free radical (DPPH), mangiferin (1,3,6,7-tetrahydroxyxanthone C2-β-D-glucoside), quercetin 3-β-D-glucoside, purity ≥ 90% HPLC grade (3,3′,4′,5,7-pentahydroxyflavone 3-β-D-glucoside), and (+)-α-tocopherol were provided by Sigma–Aldrich (Steinheim, Germany). The organic solvents ethanol, methanol and acetic acid, all HPLC gradient grade, were provided by Panreac (Barcelona, Spain). The water used in all experiments was double-distilled milliQ grade.

2.2. Extraction procedure with solvents at high pressures

Extraction tests were carried out in a high pressure apparatus supplied by Thar Technology (Pittsburgh, PA, USA, model SF100). A schematic diagram of the equipment used in this work is shown in Fig. 1. This set-up included an extraction vessel (capacity of 100 mL) with a thermostatic jacket to control the extraction temperature, two pumps with a maximum flow rate of 50 g/min (one for carbon dioxide and the other for co-solvent), a back pressure valve regulator to control the system pressure, and a cyclonic separator to allow periodic discharge of the extracted material during the extraction process. For all tests the extraction vessel was loaded with approximately 15 g of sample. Extracts were recovered in a cyclonic separator and then collected in glass bottles and stored in the extraction solvent in darkness at −20 °C prior to assay. The global yield (X_0) for all extraction method was calculated considering the ratio between mass of extract and mass of dry raw material.

A preliminary study was conducted in order to improve the yield and antioxidant activity of the extracts obtained using CO_2 and CO_2 plus co-solvents. The effects of different variables on the extraction process were analyzed by considering the following operating conditions: pressures of 10 and 40 MPa, temperatures of 35 and 55 °C, co-solvent percentages of 0 and 20% and type of co-solvent, methanol and ethanol. All tests were carried out with a CO_2 flow rate of 20 g/min and an extraction time of 3 h.

Results were compared with SWE. This technique is less dependent on pressure and highly dependent on temperature [19,20]. However, it is important to consider that the temperatures above 100 °C could generate unwanted oxidative processes [22], thus SWE tests were carried out at 100 °C, 4 MPa, a flow rate of 10 g/min and 3 h.

For this preliminary study mango leaves of the variety Osteen were used as raw material so it is the variety widely cultivated in the region of Málaga, Spain.

2.3. Antioxidant activity assay with DPPH

Antioxidant activity of extracts and standard compounds ((+)-α-tocopherol, mangiferin and quercetin 3-β-D-glucoside) was determined by 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay. The method employed was designed having in account the methods described by Brand-Williams and Scherer and Godoy [33,34]. About 0.1 mL aliquots of methanolic solutions of the samples or standards at different concentrations were each added to 3.9 mL of a 6×10^{-5} mol/L DPPH methanolic solution. The absorbance of DPPH was monitored spectrophotometrically at 515 nm at 0 min and every 2 min until the reaction reached the steady state. The DPPH concentration (C_{DPPH}) in the reaction medium was calculated from a calibration curve determined by linear regression with Eq. (1):

$$\text{Abs} = 12,709 \cdot C_{DPPH} + 0.002 \quad (1)$$

The percentage of DPPH remaining was calculated as described in Eq. (2)

$$\%\text{DPPH remaining} = \frac{C_{DPPH_t}}{C_{DPPH_0}} \times 100 \quad (2)$$

The EC_{50} (efficient concentration providing 50% inhibition) was calculated graphically using a non-linear fitting curve by plotting the sample concentration vs. the % DPPH remaining on steady state. The antioxidant activity was expressed as the Antioxidant Activity Index (AAI) which was calculated considering the final concentration of DPPH and the EC_{50} of the tested compound in the reaction as follows (Eq. (3)):

$$\text{AAI} = \frac{\text{final concentration of DPPH } (\mu\text{g/mL})}{EC_{50} \ (\mu\text{g/mL})} \quad (3)$$

The final concentration of DPPH was calculated respect to the concentration of DPPH in the reaction medium. Plant extracts showed poor antioxidant activity when AAI < 0.5, moderate antioxidant activity when AAI is between 0.5 and 1.0, strong antioxidant activity when AAI is between 1.0 and 2.0, and very strong when AAI > 2.0 [34]. The assays were carried out in triplicate. Results were compared with standards of (+)-α-tocopherol, mangiferin and quercetin 3-β-D-glucoside.

2.4. Identification and quantification of phenolic compounds by HPLC

Separation of phenolic compounds was performed using an Agilent HPLC series 1100 system (Agilent, Germany) equipped with a