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Evaluation of extraction methods for preparative scale obtention of mangiferin and lupeol from mango peels (*Mangifera indica* L.)

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ABSTRACT

Bioactive compounds have become very important in the food and pharmaceutical markets leading research interests seeking efficient methods for extracting these bioactive substances. The objective of this research is to implement preparative scale obtention of mangiferin and lupeol from mango fruit (*Mangifera indica* L.) of autochthonous and Ataulfo varieties grown in Nayarit, using emerging extraction techniques. Five extraction techniques were evaluated: maceration, Soxhlet, sonication (UAE), microwave (MAE) and high hydrostatic pressures (HHP). Two maturity stages (physiological and consumption) as well as peel and fruit pulp were evaluated for preparative scale implementation. Peels from Ataulfo mango at consumption maturity stage can be considered as a source of mangiferin and lupeol using the UEA method as it improves extraction efficiency by increasing yield and shortening time.

1. Introduction

Mango fruits are considered a source of bioactive compounds used as processing aids for preventing diseases and for providing wellbeing and health. Recently, various compounds with bioactive properties have been identified. These compounds have attracted the attention of both, consumers and the scientific community because the epidemiological evidence have demonstrated the benefits of consumption in the prevention of various human diseases (Berardini et al., 2005).

Nayarit, Mexico is considered as one of the leader producers of several varieties of mango, with a portion of the production destined to industrialisation. The mango products industry generates high amounts of byproducts and their removal of represents both, a cost to the food processor and a negative impact on the environment. The research in the last 20 years has revealed that many of these byproducts could serve as a potentially valuable source of bioactive compounds. Despite of this, the vast majority of byproducts are not currently exploited mainly due to the lack of adequate techniques for extraction at industrial scale.

Various extraction techniques can be applied for obtaining bioactive compounds. Classical techniques for extracting nutraceuticals with solvents from plant matrices are based on the choice of solvent coupled with the use of shaking and/or heat. The traditional methods of extraction include Soxhlet extraction procedure and maceration, however, they are often very time consuming, require relatively large amounts of solvents as well as prolonged periods of operation, causing a possible negative effect on the activity of these compounds (Dorta, Lobo, & Gonzalez, 2012).

According to Gao and Liu (2005) emerging extraction methods are based on improving the efficiency of traditional methods which employ physical action on the material.

Some of these techniques represent a reduced impact on the environment by using shorter times and lower quantity of solvents. Among these techniques, the most notable are microwave assisted extraction (MAE), ultrasound-assisted extraction (UAE), and extraction by high hydrostatic pressure (HHP). These methods are known as unconventional methods and they are characterised as fast and efficient for extraction of analytes from solid matrices. These techniques have been used to extract bioactive compounds from various plant materials such as olives (Sánchez Ávila, Priego Capote, & Luque de Castro, 2007), rosemary (Yokozawa et al., 1998) and green tea leaves (Pan, Niu, & Liu, 2003).

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Mangiferin (1,3,6,7-tetrahydroxyxanthone-2-glucopyranoside) is a c-glucoside xanthone, identified in high concentration in mango fruit (Acosta-Esquijarosa, Nuevas-Paz, Amaro-González, & Álvarez-León, 2009). Some authors have studied various biological actions, confirming the bioactivity of xanthones (Carvalho et al., 2007; Muruganandan, Gupta, Kataria, Lal, & Gupta, 2002; Rajendran, Ekambaram, & Sakthisekaran, 2008). Lupeol, present in mango fruit (Siddique & Saleem, 2011), (20 (29)-Lupen-3-beta-ol) is a pentacyclic triterpene with pharmacological activity against various diseases. Both bioactive compounds have beneficial effects against diseases such as inflammation (Fernández, Álvarez, García, & Sáenz, 2001), arthritis, diabetes, cardiovascular ailments (Al-Rehaily, El-Tahir, Mossa, & Rafatullah, 2001), renal disorder, hepatic toxicity, microbial infections (Sudhahar, Veena, & Varalakshmi, 2008), and cancer (Chaturvedi, Bhui, & Shukla, 2008).

These compounds have been extracted from the mango fruit by conventional methods and extraction yield has generally been low (1–2%), and depended on the extraction conditions (Diouf, Stevanovic, & Boutin, 2009; Ku, Jang, & Mun, 2007; Vázquez, González-Álvarez, Freire, López-Suevos, & Antorrena, 2001).

Therefore, the objective of this research was to implement preparative scale obtention of mangiferin and lupeol from autochthonous and Ataulfo varieties of Mango fruit (*Mangifera indica* L.) grown in the state of Nayarit, using emerging extraction techniques.

2. Materials and methods

2.1. Biological material

Two varieties of mango fruit, Ataulfo and autochthonous, were used. Two maturity states were used, physiological and consumption, based on TSS (total soluble solids), (physiological maturity 9–10 TSS and consumption maturity 12–15 TSS). Mango fruits were then selected and washed with tap water.

Samples from the two varieties of mango were manually peeled and chopped, and pulp and peels were obtained. Samples were freezed at 70 °C, followed by freeze-drying at 50 °C and 0.12 MBar using a FreeZone 4.5 freeze Dryer until obtaining 3% (dry basis) moisture was achieved (24 and 48 h for the peels and pulp, respectively).

2.2. Extraction of bioactive compounds

Mangiferin was extracted using an ethanol–water (8:2 v/v) solution as extraction solvent and to extract lupeol, the solvent hexane was used, both at a ratio of 1:10 (g sample:ml solvent).

2.2.1. Maceration extraction

Cold maceration was done by placing 10 g of freeze-dried sample in contact with the solvent in an Erlenmeyer flask, and then agitated on an orbital shaker (BOEKEL/290400) at a constant agitation speed (200 rpm) according to Aspé and Fernández (2011) and controlled temperature of 25 °C for 24 h.

2.2.2. Soxhlet extraction

Continuous Soxhlet (NOVATECH) extraction was performed by successive washing for 8 h. 230 ml of solvent was placed in a ball flask, whereas 10 g of freeze-dried sample was placed in the extraction chamber (Aspé & Fernández, 2011; Bimakr et al., 2011).

2.2.3. Sonication extraction (UAE)

10 g of freeze-dried sample was placed in contact with the solvent at 25 °C according to Aspé and Fernández (2011), in a Branson Sonicator model 1510. The sample was treated for 30 min at a constant frequency of 42 kHz.

2.2.4. Microwave extraction (MAE)

Flasks with 10 g of freeze-dried sample in contact with solvent were exposed to the radiation in a microwave chamber (SHARP R-320BG), operating at 600 W. The extraction was carried out for 1 min in 30 s irradiation cycles and 10 min of cooling to maintain a temperature of 25 °C (Aspé & Fernández, 2011; Padmapriya, Dutta, Chaudhuri, & Dutta, 2012).

2.2.5. High hydrostatic pressure extraction (HHP)

The application of high hydrostatic pressure was carried out in an autoclave by Avure Autoclave Systems, Erie, PA, USA, model CIP42260, equipped with a pressurizing chamber with an inner diameter of 101.6 mm and a length of 584.2 mm, operating at a maximum capacity of 350 MPa, and equipped with a Sullair compressor with a maximum air speed of 125 PSI. A mixture of 5:1 (water:anti-corrosive lubricant) was used as pressure fluid. The extractions were performed to 10 g of freeze-dried sample in contact with the solvent (1:10) at 25 °C (Corrales, García, Butz, & Tauscher, 2009), with a pressure of 150 MPa for 20 min (optimal conditions resulted from previous experiences (results no showed).

2.2.6. Extract concentration

The whole extracts were filtered through Whatman No.1 filter paper and concentrated on a Buchi rotary evaporator, model R-205, at a temperature of 40 °C at 300 rpm to obtain a solvent-free extract.

2.3. Quantitative determination of bioactive compounds

The bioactive compounds of interest were quantified using the external standards for mangiferin and lupeol (Sigma–Aldrich, USA, 95%) using a Waters HPLC system Model 1525 equipped with dual pump and an UV detector. The separation was performed in a 5 μm and 250*4.6 mm C-18 Thermo Scientific column. An injection volume of 10 μl and an elution rate/flow of 1 ml/min were used.

The mobile phase used for mangiferin was A: 3% acetic acid and B: acetonitrile. The mobile phase was applied in a gradient program as follows: 5 min 10% B, 15 min 80% B and 3 min 100% B. The UV wavelength was 254 nm. The mobile phase used for lupeol, was methanol at a rate of 90 ml/min for 15 min, with detection at 210 nm.

2.4. Data analysis

Statistical analysis was performed using the software SAS (*Statistical Analysis System*, version 9.0 for Windows). Data were analysed using an ANOVA followed by a LSD test ($P < 0.05$).

3. Results and discussion

3.1. Quantification of mangiferin and lupeol in components of mango fruit

Mangiferin and lupeol contents in autochthonous and Ataulfo varieties of mango fruit in the pulp and peels at two maturity stages obtained using the sonication extraction method were quantified (Fig. 1). Chromatograms obtained for mangiferin and lupeol showed that mangiferin is the compound found and extracted in higher concentration in mango fruit compared to lupeol concentration (Fig. 1a and b). This fact has been previously referred (Berardini et al., 2005 and Barreto et al., 2008). Chromatograms (Fig. 1a and b) reflect the existence of other compounds with