



Combining pressurized liquids with ultrasound to improve the extraction of phenolic compounds from pomegranate peel (*Punica granatum* L.)

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ABSTRACT

The combination of ultrasound and pressurized liquid extraction (UAPLE) was evaluated for the extraction of phenolic compounds from pomegranate peels (*Punica granatum* L.). The influence of several variables of the process on extraction yield, including solvent type (water, ethanol + water 30, 50 and 70% v:v), temperature (50–100 °C), ultrasound power (0–800 W at the generator, or 0–38.5 W at the tip of the probe), mean particle size (0.68 and 1.05 mm), and number of cycles (1–5), were analyzed according to the yield of 20 different phenolic compounds. The most suitable temperatures for the extraction of phenolic compounds using water were from 70 to 80 °C. In general, 100 °C was not adequate since the lowest extraction yields were observed. Results suggested that ultrasound had a greater impact on extraction yields using large particles and that intermediate ultrasound power (480–640 W at the generator, or 23.1–30.8 W at the tip of the probe) produced the best results. Using small particles (0.68 mm) or large particles (1.05 mm), extraction with ultrasound was 1 cycle faster. Ultrasound may have offset the negative effect of the use of large particles, however, did not increase the yield of phenolic compounds in any of the cases studied after five cycles. Additionally, the continuous clogging problems observed with small particles were avoided with the use of large particles, which combined with ultrasound allowed consistent operation with good intra and inter-day reproducibility (> 95%). Using samples with large particle size, the best extraction conditions were achieved with water extraction solvent, 70 °C extraction temperature, ultrasound power at 480 W, and 3 cycles, yielding 61.72 ± 7.70 mg/g. UAPLE demonstrated to be a clean, efficient and a green alternative for the extraction of phenolic compounds from pomegranate peels. These findings indicate that UAPLE has a great potential to improve the extraction of bioactive compounds from natural products.

1. Introduction

Among bioactive compounds in natural products, phenolic compounds are one of the most studied classes due to their association with several positive effects on health and disease prevention. They are found in high concentrations in several fruits such as apples, grapes, and pomegranate [1]. Pomegranate peels are an interesting source of phenolic compounds, since it represents 38% of total fruit weight [2] and contains more phenolic compounds than pomegranate seeds and pulp, showing potential to be explored as a functional ingredient [3]. Several phenolic compounds, including anthocyanins, hydrolyzable tannins, and hydroxybenzoic acids have been identified in pomegranate

peels, where ellagitannins are the predominant phenolic class and punicalagin (HHDP-gallagyl-hexoside) is the main compound present [4]. Studies have shown that pomegranate peels possess anti-inflammatory [5], threatening maladies such as cancer [6], type 2 diabetes [7] and cardiovascular diseases [8,9].

However, in order to be able to use them as therapeutic agents or to be able to analyze their concentration in foods, it is necessary to remove them from the raw material/sample matrix, which can be accomplished by different techniques and conditions. There is a renewed interest in the development of efficient extraction methods that are faster, cheaper and more environmentally friendly than conventional techniques available while minimizing degradation of target compounds. There are

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several extraction techniques that are being studied to improve the extraction of bioactive compounds from natural products, including pressurized liquid extraction (PLE), supercritical fluid extraction (SFE) and ultrasound-assisted extraction (UAE) [1].

PLE is an extraction technique where temperature and pressure are used to accelerate extraction of compounds from solid and semi-solid samples. Pressure has been reported to help driving the solvent into the pores of the matrix and enhance the analyte solubility [10]. Since PLE is conducted at elevated pressures, it allows liquid extraction at temperatures above the boiling point of the solvent at atmospheric pressure, thereby improving analyte solubility and its desorption from the matrix. Moreover, the temperature can dramatically modify the relative permittivity of the extracting fluid, increasing its selectivity [11]. Pressurized solvent techniques also offer the advantage of enhanced target molecule specificity and speed due to physicochemical properties of the solvent, including density, diffusivity, viscosity and dielectric constant, which can be affected by the pressure and temperature of the extraction system [12].

PLE has been successfully used for the extraction of different classes of phenolics from the most diverse samples including *juçara* [13], blueberry [14], rice grains [15], and black bamboo leaves [16]. It also has been used for the extraction of phenolics from the peels of potato [17], pomegranate [18,19], blackberry [20] and jaboticaba [21], among others. In general, PLE provides excellent results for the extraction of phenolics from natural products.

UAE is based on the formation of longitudinal ultrasonic waves capable of causing the phenomenon of cavitation due to the formation of expansion and compression regions. The energy accumulation due to compression and expansion cycles causes the collapse of gas bubbles, and a resulting shock wave passes through the solvent, exerting a mechanical effect that facilitates its penetration and affects the cell walls of the matrix, resulting in the release of large amounts of intracellular content to the extraction medium [1,22]. There are few studies that use UAE to extract phenolics from pomegranate peels [23] among other types of samples, as soybean [24], blueberry [25], and mulberry [26]. Usually, UAE is capable to accelerate the extraction of phenolic compounds, improve the process and produce better results than conventional techniques.

PLE and UAE are alternatives to conventional techniques and may increase extraction efficiency while contributing to environmental preservation by reducing the use of organic solvents or by replacing them with “green” solvents, such as water. These innovative techniques are also considered “green” extraction techniques because they can reduce the energy consumed in the process leading to lower environmental impacts [27].

Additionally, an interesting feature of UAE is the possibility of combining it with other techniques, such as microwaves, solid phase extraction and supercritical fluid extraction [28]. Studies suggest a positive effect of ultrasound in SFE process in terms of efficiency, selectivity and yield on the extractions of blackberry antioxidant compounds [22] and pepper capsaicinoids [29]. The mentioned effects are related, at least in part, by increased mass transfer caused by the application of ultrasound in a supercritical medium.

However, these studies use only the combination of ultrasound with SFE. Due to the technical similarities between SFE and PLE in terms of equipment, it is feasible to assume that combination of UAE and PLE is possible and with great potential to be explored. The combination of these technologies could be explored in small-scale processes, such as the sample preparation for the analysis of bioactive compounds in natural products and in a semi to preparative scale separations. Furthermore, developments in ultrasonic equipment are such that it is also feasible to consider commercial opportunities based on the industrial-scale ultrasonic aided extraction of bioactive compounds, with worthwhile economics gains [30].

Despite the great potential, there are no applications of ultrasound combined with PLE in the scientific literature to the present time.

Therefore, it is necessary to study how process variables (temperature, solvent, ultrasound power, pressure, particle size) interact and affect the extraction of bioactive compounds in natural products. In this context, this work evaluated the possibility of combining two extraction techniques (UAE + PLE = UAPLE) to improve the efficiency, selectivity, and yield of the extraction of phenolic compounds from pomegranate peel and the influence of the most important variables in the process.

2. Materials and methods

2.1. Solvents and reagents

Ethanol, methanol, acetonitrile, phosphoric and formic acid were purchased from Labsynth (Diadema, SP, Brazil). Ultrapure water was provided by an Elga Veolia Purelab Flex 3 system (High Wycombe, UK). Standards of the compounds analyzed by liquid chromatography (punicalagin and ellagic acid) were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Sample and sample preparation

Pomegranate fruits (Wonderful variety) cultivated in California (USA) were purchased at an establishment in Limeira-SP (Brazil). Samples were processed by breaking the fruit and separating the seeds/arils from the peels using a commercial pulper (Des-60 Braesi, Caxias do Sul, Brazil). Peels (exocarp and carpelar membrane) were dried at 70 °C in a forced air circulation oven (JP Selecta, Barcelona, Spain) during 48 h. After this process, samples were sieved and stored at -20 °C in amber glass vials until the preparation of the extracts. The moisture content of samples ($6.50 \pm 0.00\%$) was determined using an air convection oven held at 105 °C (Marconi, MA030/12, Piracicaba, Brazil). The particle size was determined using sieves and vibratory shaker (Bertel Metallurgic Ind. Ltda., Brazil) with the following sequential aperture sizes: 14, 18, 50, and 80 mesh (Equivalent to Tyler series, Wheeling, USA).

The mean particle diameter was calculated according to the Standards of The American Society of Agricultural Engineers [31]. To check the influence of particle diameter, samples were classified into two groups: large particles sample, formed by particles retained on a sequence of sieves of 14 mesh (15.6%), 18 mesh (83.0%) and 50 mesh (1.4%); overall size 0.297–1.410 mm, 1.05 mm mean particle size; small particles sample, formed by the material retained on a sequence of sieves of 18 mesh (49.3%), 50 mesh (38.1%) and 80 mesh (8.4%); overall size 0.177–1.000 mm, 0.68 mm mean particle size.

2.3. Ultrasound-assisted pressurized liquid extraction (UAPLE)

Extractions were performed on the EXTRACT-US system (Fig. 1) (FAPESP 2013/043044 – patent pending) using pressurized liquids assisted by ultrasound. The system is composed of a HPLC pump (PU2080 – Jasco, Japan), ternary gradient unit (LG 2080-2, Jasco), on-line degasser (DG 2080-55, Jasco), UV–VIS detector (UV-7075, Jasco), five automatic two position 10 port valves (Waters Corporation, Milford MA, USA), 10 mL stainless steel extraction cell coupled to the ultrasonic horn (800 W, 19 kHz, 13 mm of diameter, Unique group, Indaiatuba, Brazil), backpressure regulator valve (TESCOM 26-1700 Series) and block valves (Autoclave Engineers, Erie, USA).

Extraction cell packing was done adding in sequence and in a standardized way. The filter paper was placed on the bottom of the extraction cell over the stainless steel filter. Sequentially, layers of glass wool (0.3 g), glass balls of 3 mm diameter layer (5.0 g), dry pomegranate peel sample (1.0 g) and glass wool layer (0.1 g) were placed inside the extraction cell. Afterward, the extraction cell was trodden to the ultrasonic horn to start the extraction cycle. An extraction cycle consisted in filling the extraction cell with the selected extraction

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