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Ultrasound-assisted extraction of hesperidin from Penggan (*Citrus reticulata*) peel

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Abstract

Hesperidin, an abundant and inexpensive bioflavonoid in Penggan (*Citrus reticulata*) peel, has been reported to possess a wide range of pharmacological properties. Ultrasonic extraction is an effective technique for the isolation of bioactive compounds from vegetable materials. In this study, the application of ultrasonic method was shown to be more efficient in extracting hesperidin from Penggan (*C. reticulata*) peel than the classical method. The effects of main ultrasonic-assisted extraction conditions on extraction yields of hesperidin from Penggan (*C. reticulata*) peel were evaluated, including extraction solvents, solvent volume, temperature, extraction time, ultrasonic power, ultrasonic frequency. Results showed that solvent, frequency and processing temperature were the most important factors for improving the extracting yields of hesperidin. When performed at the same temperature under the same time using three frequencies, methanol as the solvent improved the extraction yield evidently compared with ethanol or isopropanol; by comparison of the frequency influence, the yield of hesperidin was higher at 60 kHz than at 20 kHz and 100 kHz. The optimum ultrasonic conditions were determined as: methanol, frequency of 60 kHz, extraction time of 60 min, and temperature of 40 °C. In addition, the ultrasonic power had a weak effect on the yields of hesperidin within the experimental range. Extending ultrasonic treatment times did not result in degradation of hesperidin; the rotary beaker for materials can increase the yields of hesperidin.

Keywords: Extraction; Ultrasound; Hesperidin; Penggan (*Citrus reticulata*) peels

1. Introduction

Citrus fruit holds a unique place in plant kingdom and occupies a resulting solitary position in the human diet. Citrus is the second largest growing fruit in China, playing an important role in food processing. Citrus peels represent a potential material for pharmaceutical and food industry since they contain significant flavonoids that are bioactive compounds with health-related properties. They have several hydroxyl in different position of rings, where there is strong chemical activity. Such components as antioxidants in various biological systems [1,2], can prevent pregnancy [3], inhibit the vitro proliferation of cancer cells [4], and display anti-allergic and anti-inflammatory activity [5]. Hesperidin is the most abundant flavanones component in Penggan peel. However, most of citrus peels in the conventional food processing as byproducts were wasted, resulting in certain environmental pollution. One of main reasons for the above fact is lack of an effective way to extract the useful components from the citrus peels.

Extraction of bioactive compounds from vegetable materials with a solvent is a classical operation applied in many industrial processes, particularly the pharmaceutical industry. It is obvious that medical interest in plants derived drugs has led to an increased need for ideal extraction methods, which could obtain the maximum of the bioactive constituents in a shortest processing time with a low cost. Ultrasonic-assisted extraction has been proved to significantly decrease extraction time and increase extraction yields in many vegetable materials [6–8]. Just as in nature,

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the application of ultrasound in the food industry can be divided into two distinct categories of low-intensity high frequency ($f > 100$ kHz) and high-intensity low frequency (20 kHz $\leqslant f \leqslant$ 100 kHz) ultrasound. Low-intensity ultrasound does not alter the physical or chemical properties of the material through which the ultrasonic wave propagates [9]. High-intensity shock wave generates intense pressures, shear and temperature gradient due to the bubble of cavitation inducing the majority of ultrasonic effects within a material, which can produce physical, chemical and mechanical effects [10]. The effect of high-intensity ultrasound on process and products which cause physical disruption or promote certain chemical reaction (e.g. oxidation) depends on many variables including reaction medium characteristics, ultrasonic parameters, ultrasonic generator performance, size and geometry of treatment tank [11,12]. The extraction of organic compounds within vegetable materials by ultrasound can significantly enhance ascribed to either acting by mechanical effects that providing a greater penetration of solvent into cellular materials resulting from the disruption of biological cell walls to facilitate the release of content [13,14], or in combination of various effects [12], but the combined effects of many variables on the power still unknown.

More recently, application of ultrasonic technology in food processing attracted widely attentions [15]. Comparative investigation of the influence of classical and ultrasonic techniques on the yield and the structural features of extracts from wheat straw and the root of valerian have shown a higher yield and stability of functional properties of lignin and water-soluble polysaccharides using the latter method [16,17]. Ultrasonic extraction carnosic acid from *Rosmarinus officinalis* using ethanol was effective in producing a greater yield and shortening of extraction time [18]. Sun and Tomkinson [19] reported a procedure for ultrasonic extraction of hemicelluloses from wheat straw, Romdhane and Gourdou [20] isolated pyrethrines from pyrethrum flowers and oil from woad seeds using ultrasound. The optimization of ultrasound variables according to a specific plant matrix is also of importance for achieving high extraction yield. In the cases of *Eucommia ulmodies* [21] and *Hibiscus tiliaceus* L. flowers [22], the optimum ultrasonic extraction conditions were established.

These earlier works have proved that ultrasonic extraction is a potential technology in food processing and pharmaceutical industry. Ultrasonic technique for hesperidin extraction has been primarily proven to be an attractive process [23,24]. However, there have not been fully reports on application of ultrasonic technique for extraction of hesperidin from Penggan peel. The objective of this paper is to evaluate the influence of main extraction conditions including solvent, temperature, frequency, and power on the yields and stability of hesperidin from Penggan (*C. reticulata*) peels.

2. Experimental

2.1. Apparatus

Ultrasonic extraction experiments were carried out in ultrasonic cleaning baths produced by Guangzhou Sonoc Ultrasonic Electronic Equipment Co. Ltd., China, which can work at 20 kHz, 60 kHz, 100 kHz frequencies with a variable power output, and has a digital timer to set up time and a temperature controller to control the temperature, a voltage meter, a electric current meter used for measuring the electrical power consumed, a rotary gear on which the beakers are placed, the beaker rotated when the rotary gear was drove by Motor. The bottom of water tank was made a shape of quadrangular frustum of a pyramid equipped with five same sonic generators on its every side. Schematic diagram of the ultrasonic apparatus is represented in Fig. 1.

The extracted hesperidin was analyzed by High performance liquid chromatographic (HPLC) in a Waters 2695-2996 system consisting of 515 pump and an Econosphere ODS-2 column of 250 × 4.6 mm dimension and a C-18 col-

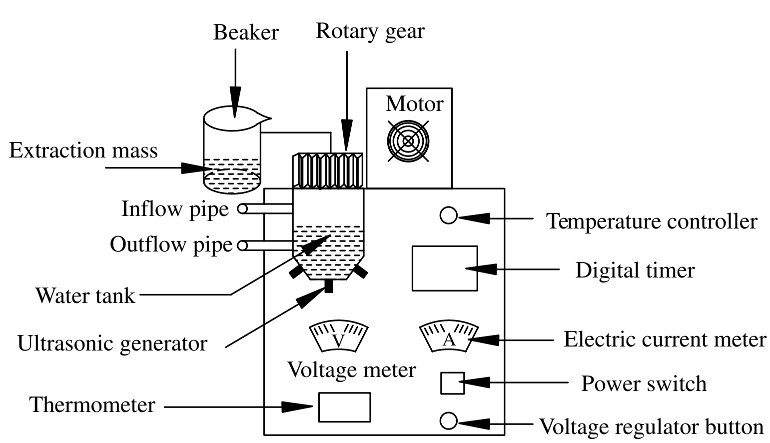

Fig. 1. Schematic diagram of the ultrasonic apparatus.

umn (250 mm × 4.6 mm, ID 5 μm) from Dilma Technologies Co. Ltd. (America).

2.2. Materials and reagents

Fresh Penggan (*C. reticulata*) were collected from Quzhou, Zhejiang province, China, in November 2005. Penggan peels were dried in an oven with air circulation at 50 °C, the dried Penggan peels were grounded in laboratory with a blade mixer to pass through a 0.45–1 mm screen and were kept in labeled capped plastic inside desiccators until use. Three different solvents: methanol, ethanol, isopropanol were used for extraction of hesperidin from Penggan peel. All chemical reagents used in experiments were of analytical-reagent grade.

Methanol (reagent for HPLC), glacial acetic acid (reagent for HPLC) and redistilled water were filtrated through a 0.45 μm membrane before use. The standard hesperidin was purchased from Sigma Aldrich Co. Ltd. All glasswares and plasticware were rinsed with deionised water.

2.3. Extraction method

The grounded powders of 1 g were first loaded into a 600 ml beaker (8 cm diameter × 14.5 cm height) sealed by plastic film to avoid loss of solvent and then extraction solvent was added with a solid–liquid ratio of 1:40. The particle size and solid–liquid ratio used in experiments were selected from the previous study [19]. The sample beakers were immersed into the ultrasonic cleaning bath for irradiation under different extraction conditions. Finally, extracts were filtered off through 0.45 μm microporous membrane and the filtrate was collected for HPLC analyses. All experiments were performed in duplicates.

2.4. Soxhlet extraction

The grounded powders of 3 g and the solvent 120 ml were put into the soxhlet apparatus. The soxhlet extraction was performed from 20 to 160 min time at 60 °C, intervals of 20 min, which was used to as a control for comparison with ultrasound-assisted extraction methods.

2.5. Ultrasonic bath extraction

Effect of absorption-uniformed irradiation power for materials on the yield of hesperidin was determined by rotary beaker with 60 kHz at 40 °C for 40 min. Materials in the following experiments were treated with rotary beaker.

The dried samples were extracted with 20 kHz, 60 kHz, 100 kHz frequencies for 20 min at 30 °C, 40 °C, 50 °C using methanol, ethanol, isopropanol. In parallel another investigation was carried by using only methanol as the extracting solvent at 40 °C. Extraction time was performed from 20 to 160 min at 40 °C, time intervals of 20 min, meanwhile, hesperidin standard solutions were treated in above ultrasonic conditions in order to test if extended ultrasonic time can result in the degradation of hesperidin. Ultrasonic power levels of 3.2, 8, 30, 56 W were investigated.

2.6. Chromatographic analysis

For HPLC analysis, the mobile phase was methanol–deionised water–glacial acetic acid (10:1:14) at a flow rate of 1 ml/min, the column temperature was 25 °C and sample volume injected was 10 μl, the optimum detecting wavelength for hesperidin was 283 nm. Under above conditions hesperidin gave a peak at 9.1 min. Fig. 2 shows the HPLC chromatograph of standard hesperidin.

3. Results and discussion

3.1. Effects of rotary beaker on the extraction yields of hesperidin from Penggan (C. reticulata) peels

Fig. 3 shows the effects of beaker types on the yield of hesperidin extracted from Penggan peels under ultrasonic conditions: the extraction time of 40 min, methanol as the solvent, a frequency of 60 kHz, and a temperature of 40 °C. It can be found that the yields of hesperidin in

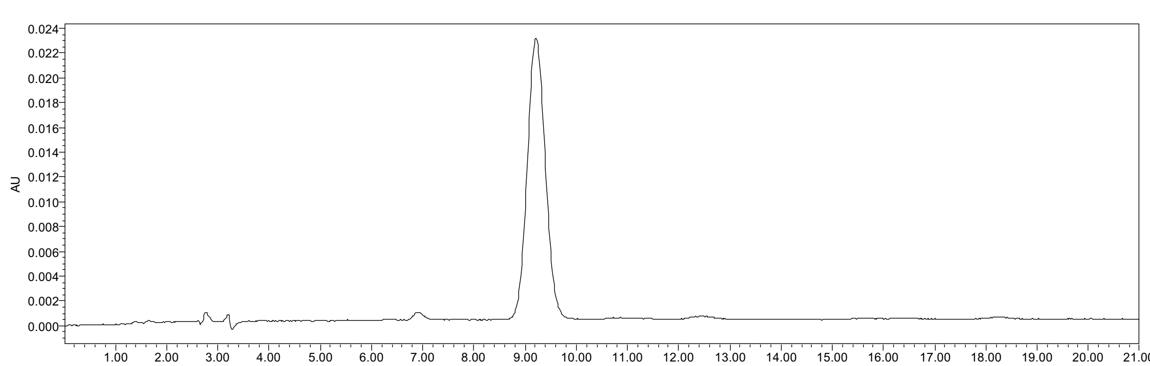

Fig. 2. The HPLC chromatograph of standard hesperidin.

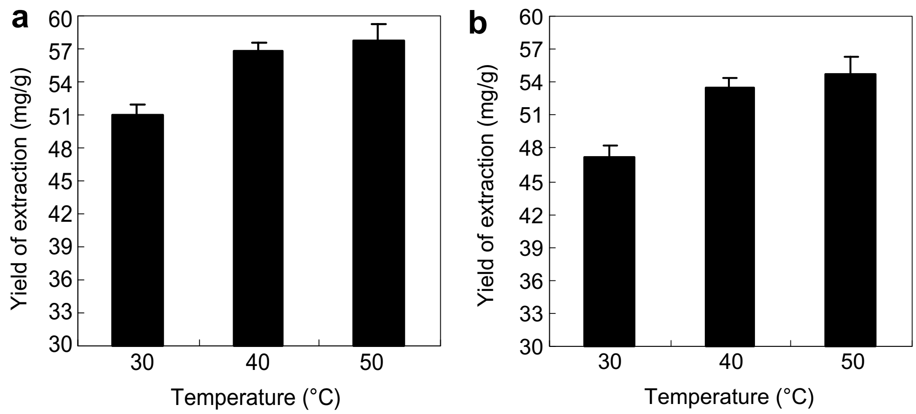

Fig. 3. Effect of rotary beaker on the extraction yields of hesperidin from Penggan (*C. reticulata*) peels using methanol with 60 kHz for 60 min at 40 °C and 30 W. (a) Rotary beaker and (b) fixed beaker.

rotary beaker were higher than those in fixed beaker. In addition, the yield of extraction increased with the rise of temperature despite of fixed and rotary beaker (Fig. 3). The result is probably linked to the samples in rotary beaker more uniformly absorption of ultrasonic power than in the latter.

3.2. Effects of solvent, temperature and frequency on the extraction yields of hesperidin from Penggan (C. reticulata) peels

The results illustrated in Fig. 4 show the yields of hesperidin are dependent on different solvents. Methanol appears to be the most effective extraction solvent under the same ultrasonic conditions followed by ethanol and isopropanol. The different yields of extracts might be caused by polarities of solvents [19,22]. In fact, the extraction efficiencies of solvents increases with the rise of temperature, and the temperature resulting in the maximum yield of hesperidin at 60 kHz is 50 °C. On the other hand, high temperature is not beneficial for ultrasonic extraction because of evaporation of solvent. So 40 °C is chosen as an optimal temperature in the following extraction procedures.

3.3. Effects of extraction time on the extraction yields of hesperidin from Penggan (C. reticulata) peels

The effect of extraction time using ultrasound and soxhlet method on the extraction yields of hesperidin from Penggan peels is represented in Fig. 5. For all cases shown, the yields of hesperidin ultrasonically assisted method under three frequencies all are higher than those with the soxhlet method. The yields of the soxhlet extraction for 160 min did not achieve those by the ultrasonic extraction under three frequencies for 20 min, even the soxhlet extraction work at a temperature of 60 °C, but ultrasonic extraction is only at 40 °C. The results suggested the advantages of ultrasound-assisted extraction, which can achieve at lower temperature and can efficiently reduce extraction time, comparison by soxhlet extraction method.

For three different frequencies, the extraction yields was significantly time-dependant and increased with extended ultrasonic times at three different frequencies, especially from 20 min to 60 min, but slowly from 60 min to 160 min. The results indicated that the efficient extraction period for achieving maximum yield of hesperidin with three frequencies was about 60 min. The similar results have been reported in the literature [25].

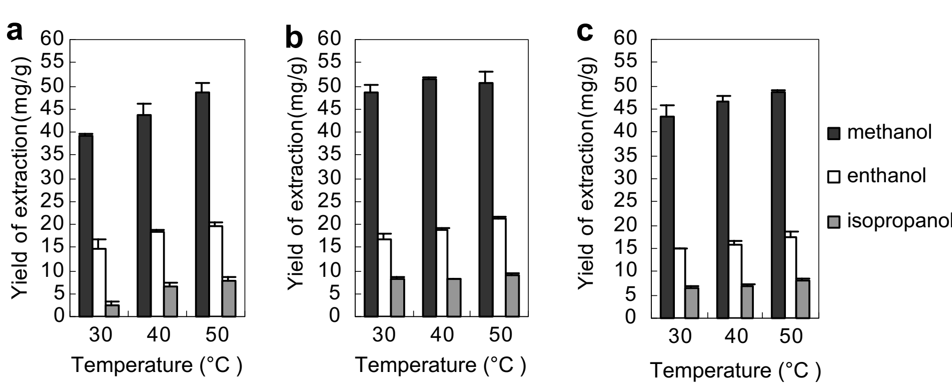

Fig. 4. Effect of solvent and frequency on the yields of hesperidin from Penggan (*C. reticulata*) peels using 30 W at different temperatures for 20 min. (a) 20 kHz, (b) 60 kHz and (c) 100 kHz.

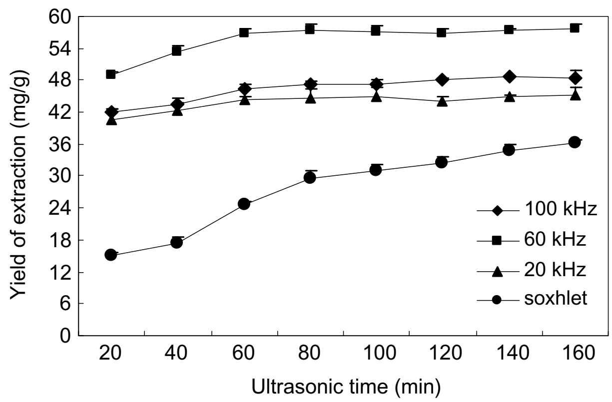

Fig. 5. Effect of extraction times on the yields of hesperidin from Penggan (*C. reticulata*) peels using methanol with different frequencies (20 kHz, 60 kHz, 100 kHz) at 40 °C and 30 W.

In order to determine the influence of the longer extraction time on the stability of hesperidin, the standard solution of hesperidin under the same ultrasonic conditions was measured. The results obtained by the HPLC analysis were shown in Fig. 6, it can be seen that the concentration of standard solution of hesperidin did not change (Fig. 6), suggesting that hesperidin under ultrasonic parameters selected in this study was not degraded. This is in agreement with earlier studies [16,17,26].

3.4. Effect of ultrasonic power on the extraction yields of hesperidin from Penggan (C. reticulata) peels

Fig. 7 shows that ultrasound has a weak effect on the yield of hesperidin according to the power consumption including 3.2, 8, 30, 56 W at different frequencies. Whenever ultrasonic energy is propagated into an attenuating material such as tissue of plant, the amplitude of the wave decreases with an increase in the distance from the horn tip [27], the volumetric energy and the mass transfer also decrease [28]. This attenuation of cavitational activity is ascribed to either absorption or scattering. Absorption represents that portion of the wave energy is converted into

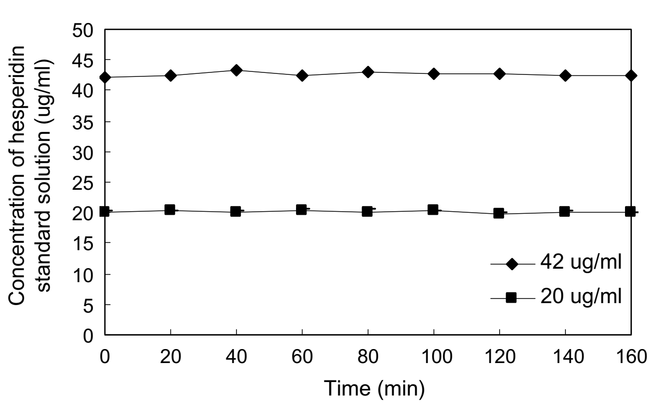

Fig. 6. Effect of ultrasonic times for the solution of hesperidin standard on the stability of hesperidin using methanol with 60 kHz at 40 °C and 30 W.

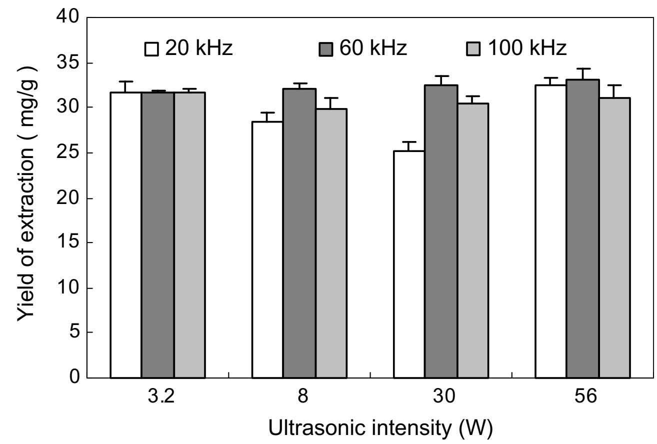

Fig. 7. Effect of ultrasonic power on the extraction yield of hesperidin from Penggan (*C. reticulata*) peels using methanol with different frequencies for 5 min at 40 °C.

heat, and scattering can be regard as that portion which changes direction. Therefore, the distance from the radiating surface can influence on ultrasound intensity, the efficient ultrasonic intensity was also measured at the vicinity of the radiating surface of the ultrasonic generator [29]. In addition, the active zone in ultrasonic horn, in which is the domains of intense chemical activity (sonochemistry) and of high mass transfer (hydrodynamics) ($f = 20$ or 40 kHz) [28], was observed in a cylinder of 6 cm in diameter [29]. In our investigation, the distance from the bottom of beaker to the ultrasonic generator was about 10 cm. Thus, the little differences of extraction yields under different ultrasonic power levels in Fig. 7 may be partially attributed to the lower ultrasonic activation due to the longer distance from the irradiating surface.

4. Conclusions

The results from this study showed that solvent in ultrasonic extraction was the most effective influence on the yield of the extracts. Both increasing temperature and extended time can enhance the efficiency of extraction. However, the selection of extraction temperature and time should carefully consider the evaporation of solvent and the solubility of compounds extracted in order to avoid loss of solvent in high temperature and reduce costs. In this paper, the most efficient ultrasonic parameters of extracting hesperidin from Penggan peels were determined as: methanol, the frequency of 60 kHz, extraction time of 60 min, temperature of 40 °C. The extended ultrasonic time does not result in the degradation of hesperidin. The ultrasonic power has a weak effect on the yield of hesperidin, because the presence of a dispersed phase contributes to ultrasound wave attenuation and the active part of ultrasound inside the extractor is restricted to a zone located in the vicinity of the emitter. In addition, the samples of absorption-uniformed irradiation power contribute to higher yields of extraction. The effect of ultrasonic on extraction the yield of hesperidin depends on many ultrasonic parameters that produce physical, chemical or mechanical effects, which will play an important role for isolation bioactive compounds from Penggan peels by individually or in combination, which is difficult to determinate the interaction of many parameters.

The practical absorption power of plant materials and a weak power effect implies to consider the active zone and reasonable distribution of ultrasonic power, which might be beneficial for potentially industrial production.

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