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Evaluation of the effect of ultrasonic variables at locally ultrasonic field on yield of hesperidin from penggan (*Citrus reticulata*) peels ☆

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ABSTRACT

It has been reported that the maximum ultrasonic power depended on the distance of ultrasonic irradiation surface. Therefore, to confirm this point, an experiment for ultrasound-assisted extraction (UAE) of hesperidin from penggan peels at locally ultrasonic field was performed by response surface methodology (RSM). A three-level three-factor Box–Behnken design was applied to evaluate the effects of three independent variables including ultrasonic power, extraction time and temperature on the yields of hesperidin at high and low ultrasonic irradiation surface. The results showed that the coefficients of two mathematical-regression models by means of the second-order polynomial equation obtained at high and low ultrasonic irradiation surface was 0.9742 and 0.9745, respectively, thus indicating that quadratic polynomial model could be used to estimate the ultrasound-assisted extraction of hesperidin. By comparison of the ultrasonic irradiation surface influence, the yield of hesperidin obtained at low ultrasonic irradiation surface was much higher than the high ultrasonic irradiation surface. Moreover, the scanning electron microscopy (SEM) showed that the particles' microstructures of Penggan peel obtained at low ultrasonic irradiation surface were destroyed more heavily than high ultrasonic irradiation surface. As a result, the vicinity of ultrasonic irradiation surface can generate stronger cavitation energy.

1. Introduction

The extraction of active ingredients from vegetal materials has a long history all over the world. More recently, with the rapid development of pharmaceutical, cosmetics and food industries, making fully use of naturally bioactive products related to healthy properties have become popular. Simultaneously, finding new ways to effectively extract these bioactive components also are concerned increasingly. Moreover, the extraction efficiency is the most crucial for industrial processing, influenced by many factors such as solvent, solvent to solid ratio, extraction time, extraction temperature, extraction method (Cacace & Mazza, 2002; Vagiri, Ekholm, Andersson, Johansson, & Rumpunen, 2012; Wettasinghe & Shahidi, 1999). Therefore, it is necessary for selection of effective extraction method and optimization of extraction factors in the practical application of industrial production, which can be obtained by empirical or statistical methods.

UAE has been widely applied in extraction of a variety of biologically active compounds from plants. In ultrasonic extraction experiments, ultrasonic parameters including solvent, particle size, frequency, ultrasonic power, extraction time and temperature are measured frequently (Capelo, Maduro, & Vilhena, 2005; Tian, Xu, Zheng, & Martin Lo, 2013), Even ultrasonic devices (ultrasonic bath or probe) have an effects on the activities of cavitation bubbles. However, the most important criterion in the classification of ultrasonic applications is the energy amount of the generated sound field (Knorr, Zenker, Heinz, & Lee, 2004). Positive or negative effects when applied ultrasonic power can be observed in different reports. For example, a positive result by Zhong and Wang (2010) showed that the higher extraction efficiency of longan polysaccharides at higher ultrasonic power was obtained. In the negative cases of extraction oil from woad seeds (Romdhane & Gourdon, 2002), the yields of oil at three levels of ultrasonic power (60 W, 100 W, 170 W) were practically unchanged.

Power ultrasound has been regarded as considerable potential for industry process for many years. Measuring ultrasonic power by different methods has been reported (Romdhane, Gourdon, &

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Casamatta, 1995), which gave the similar results that maximum ultrasonic power was observed at the vicinity of irradiating surface of ultrasonic transducer, and the decrease of ultrasonic power increased with the distance between ultrasonic irradiating surface and ultrasonic transducer. This is in accordance with a visual experiment for determination of ultrasonic power using aluminum foil in application of ultrasonic horn (Laborde, Bouyer, Caltagirone, & Cérard, 1998). However, the attenuation of ultrasonic power with increase of distance of ultrasonic irradiating surface was mostly investigated in theory. In our previous studies (Ma et al., 2008), ultrasonic power has a weak effect on the yields of hesperidin from Penggan peels, the assumption of which was resulted in the longer distance between treated materials with the irradiating surface. Therefore, it is necessary to confirm the assumption by experimental data.

It is well-known that citrus fruits have been widely used in traditional Chinese medicines (Chinese Pharmacopoeia Edition Committee, 2005), which was named chenpi (dried citrus peel). Flavonoids were recognized as the primary biological compounds. In south China, Penggan is one of the most popular citrus varieties cultivated, and Penggan peel are rich in flavonoids, specially, hesperidin is the dominant flavonoid in Penggan (it is also called as Ponkan) peel (Zhang et al., 2014), which has been reported to possess a wide range of pharmacological properties. Thus they are very beneficial to human health.

Response surface methodology (RSM) has been successfully used to optimize the critical extraction parameters by estimating interactive and quadratic effects (Sahin, Aybastıer, & Esra, 2013; Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2008). Box-Behnken design (BBD) is a suitable for response surface methods because it can reduce the number of effective experiments and indicate the role of each parameter (Liu, Mei, Wang, Shao, & Tao, 2014). BBD is used to determine the best combination of the variables for the response. In the paper, the effect of ultrasonic power on the extraction yields of hesperidin from Penggan peel at the different height of ultrasonic irradiation surface was performed by response surface methodology. The objection of the paper, as an example of extraction hesperidin, is to employ RSM to optimize the hesperidin extraction process from Penggan peel for maximum yield, and by comparison of the extraction yields of hesperidin under different ultrasound conditions, it is helpful to confirm that the activities of ultrasonic power depend on the distance of ultrasonic irradiation surface. Meanwhile, the locally ultrasonic irradiation effect on the extraction was evaluated by scanning electron microscopy (SEM) analysis in the aspect of Penggan peel particles' cellular structures.

2. Experimental

2.1. Apparatus

Ultrasonic extraction experiments were carried out in ultrasonic cleaning baths produced by Guangzhou Sonoc Ultrasonic Electronic Equipment Co. Ltd. (Guangzhou, China). A frequency of 20 kHz was selected. A variable power output, a digital timer, a temperature controller and a voltage meter were designed; an electric current meter was used for measuring the electrical power consumed. The bottom of water tank was made a shape of quadrangular frustum of a pyramid equipped with five same sonic generators on each side. The schematic diagram of the ultrasonic apparatus is described in previous paper (Ma et al., 2008). High performance liquid chromatographic (HPLC) was performed in a Waters 2695-2996 system (Waters, Milford, MA , USA) consisting of 515 pump and an Econosphere ODS-2 column of 250 × 4.6 mm dimension and a C-18 column (250 mm × 4.6 mm, ID 5 um) from Dikma Technologies Co. Ltd. (Dalian, China).

3. Materials and method

3.1. Materials and reagents

Fresh Penggan (*C. reticulata*) were provided friendly by Zhejiang Citrus Research Institute in Tai-zhou city, Zhe-jiang province, China. Penggan (*C. reticulata*) peels were dried in an oven with air circulation at 50 °C, the dried Penggan (*C. reticulata*) 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.

Methanol was used to extract the hesperidin from Penggan peels. All chemical reagents used in experiments were of analytical-reagent grade (Dingguo biotechnological Co. Ltd., Beijing, China). Methanol (reagent for HPLC), glacial acetic acid (reagent for HPLC) and redistilled water were filtrated through a 0.45 um membrane before use. All HPLC reagents and the standard hesperidin were purchased from Sigma Aldrich Co. Ltd (St. Louis, MO, USA).

3.2. Extraction method

A 600 ml flask (8 cm diameter × 14.5 cm height) named as high irradiation surface and a specialized design flask (8 cm diameter × 26 cm height) named as low irradiation surface were used in extraction hesperidin, and the height of 28 cm in Fig. 1A refers to that of ultrasonic water bath. The detailed diagram of the apparatus has shown in Fig. 1. Two flasks were made by the same glass material and have the same thickness of flask wall according to our experiment demands, which were supplied by Zhejiang university chemical plant, Hangzhou, China.

The grounded powders of 1 g were loaded into flask sealed by plastic film to avoid loss of solvent and then extraction solvent was added with a solid–liquid ratio of 1:40. The sample flask was immersed into the ultrasonic cleaning bath for irradiation under different ultrasonic conditions in Table 2. Finally, extracts were filtered off through 0.45 um microporous membrane and the filtrate was collected for HPLC analyses. All samples were prepared and analyzed in triplicate, and the results were the averages of triplicate analyses.

3.3. Chromatographic analysis

The yields of hesperidin were determined according to previous method described in Ref (Nagy, Shaw, & Veldhuis, 1977) with some modification. Prepared extracts solution was filtered through a millipore membrane (0.45 μm) before injection. The mobile phase was 100% methanol (A) and 4% (by vol) acetic acid in water (B) (A:B = 37:63) at a flow rate of 1 ml/min, the column temperature was 40 °C and sample volume injected was 10 μL. The optimum detecting wavelength for hesperidin was 283 nm. Hesperidin concentration (expressed as mg/g DW) was calculated by an external standard method using calibration curves. Standard stock solution with varying hesperidin concentrations were prepared, within the range of 1–35 μg/mL the equation of linear regression was good with $R^2 > 0.998$ for all measured hesperidin. The repeatability of intraday analysis ranged from a relative standard deviation (RSD) of 0.17% to an RSD of 1.89% ($n = 3$). The detection limits and the quantification limits were 0.042 and 0.175 μg/mL, respectively.