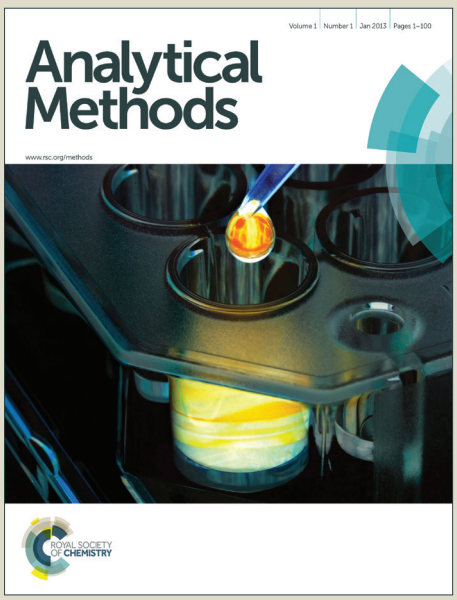
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

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ARTICLE TYPE www. rsc.org/xxxxxx | XXXXXXXX

A novel on-line ultrasonic extraction system for determination of optimal ultrasonic frequency for plant material

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Received Xth XXXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
First published on the web Xth XXXXXXXXXX 200X
DOI: 10.1039/b000000x

Ultrasonic extraction efficiency or yield varies with the change of ultrasonic frequency, because each kind of plant material possesses a unique natural resonant frequency. Only when the ultrasonic frequency is equal to the natural resonant frequency of plant material, extraction efficiency or yield will be up to the highest value. Existing ultrasonic extraction technologies or devices may be difficult to determine the optimal ultrasonic frequency for different plant material because of selecting only one or a few frequency points. To determine the optimal ultrasonic frequency, this paper presented a novel ultrasonic extraction technology in our laboratory, an on-line extraction system for determination of optimal ultrasonic frequency well suited for various plant materials. This system was composed of eight similar working groups which the ultrasonic frequency band was set in a range of 18-82 kHz. A determination method for optimal ultrasonic frequency was carried out by two steps including determining optimal frequency band and frequency point, respectively. In order to evaluate performances of this extraction system, a comparative experiment of hesperidin from tangerine peels was also performed under the same extraction conditions. Results showed that the highest extraction efficiency of extracting hesperidin from tangerine peels appeared at 47.5 kHz, which gave a higher extraction yield compared with the existing ultrasonic extraction technology, and also significantly shortened the extraction time.

1 Introduction

In resent years, there has been tremendous increase in the use of ultrasound for the extraction of plant materials, because the application of ultrasound-assisted extraction offers many advantages including higher extraction yields, lower temperature, short time and the reduction of solvents when compared to other extraction techniques.[1–3]

Many studies of a variety of substances extraction process using ultrasound have been reported.[4–7] It could be find that those studies on ultrasound-assisted extraction technology mainly focused on optimum conditions including extraction time, temperature, the type of solvent, solid/solvent ratio, electrical acoustic intensity, etc. However, the effect of optimal ultrasonic frequency on extraction efficiency has not been systematically investigated so far. Moreover, little work has been done to find the best method for ultrasonic frequency used in biological extraction.

In fact, the ultrasonic frequency will have a strong effect on extraction efficient. During the extracting process, not all bubbles are capable of producing significant cavitation effects. The greatest coupling of the ultrasonic energy will occur when the natural resonance frequency of the bubbles is equal to the ultrasonic frequency .[8,9] The extraction bubble natural resonance frequency equation was deduced.[10] It indicates that if ultrasonic frequency is less than the natural resonant frequency of the bubbles, extraction yield will increase as the ultrasonic frequency increase. On the contrary, extraction yield decreases with increasing ultrasonic frequency. The research suggested that under the conditions described to study ultrasonic extraction, the yield of extraction in liquids could reach a maximum value at an optimal ultrasonic frequency because the ultrasonic frequency helped to promote bubbles collapse by driving the bubbles into resonance. Therefore, determining optimal ultrasonic frequency for various plant materials, which can obtained a maximum yield of extraction, is a very important research subject.

Some researchers have studied the ultrasonic extraction processes for various plant materials,[11–17] which could be described as follow: Firstly, ultrasonic frequencies were empirically selected from ultrasonic apparatus, then, plant materials were irradiated by selected ultrasonic frequencies. As a result, a large number of experimental data were produced. Finally, by comparing and analyzing those obtained data, the optimal ultrasonic frequencies were finally determined. However, the extraction process might result in blindness for determining optimal ultrasonic frequency because no one knows what is the ultrasonic frequency in advance. Moreover, the optimal frequency was probably not the best suitable ultrasonic frequency due to taking roughly only a few frequency points. In addition, those extraction technologies not only waste a lot of

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manpower, financial and material resources, but also can not form a stable large scale processes. Hence, research higher efficient extraction technology for determining optimal ultrasonic frequency is extremely urgent.

This paper aims to explore a highly efficient extraction system for plant materials by determining optimal ultrasonic frequency method. To achieve a on-line displaying function on a computer, and so establish a simple method for the determination, An UV transceiver with a pair of ultraviolet emitter and receiver was designed to real-time detect the extraction efficiency. In addition, in order to evaluate the performance of our novel ultrasonic extraction system, a comparison of the extraction yield of hesperidin from tangerine peels between our extraction system and an existing ultrasonic extraction technology was also carried out under the same extraction conditions. The findings of the present study may suggest a new strategy to enhance the extraction efficiency for plant materials.

2 Extraction system

The ultrasonic extraction system is composed of a computer and eight similar working groups that are constituted by various parts including a treatment tank, a signal converter, an ultrasonic generator and controller, a transducer actuator, an U-V transceiver, a peristaltic pump and a temperature controller. The schematic diagram and photograph of the extraction system are shown in Fig. 1.

Taking into account the fact that ultrasonic frequencies used for extracting plant materials are mainly concentrated close to the low frequency (20 kHz-100 kHz), in our investigation, the ultrasonic frequency band of this extraction system was set in a range of 18-82 kHz. Namely, the frequency bandwidth in the first working group was 18-26 kHz, the second one was work at 26-34 kHz, and the eighth one was set at 74-82 kHz. Of course, the frequency bandwidth of each working group could be adjusted by replacing transceivers according to different extraction results or plant materials.

An UV transceiver with a pair of ultraviolet emitter and receiver is one of key working units, which was equipped to real-time detect the extraction efficiency of product concentration because different ultraviolet wavelengths can be absorbed by some analytes concentrations. Fig. 2 shows a photograph of UV transceiver. To ensure the ultraviolet light emitted by UV emitter can be fully absorbed by UV receiver, The UV emitter and receiver were disposed at the same height degree and kept a distance of 6 cm. Moreover, the signal converter was used to achieve the functions of signal detection and conversion from the UV transceiver. The converted electrical signals were sent to the computer on which these signals were processed, monitored, controlled and displayed.

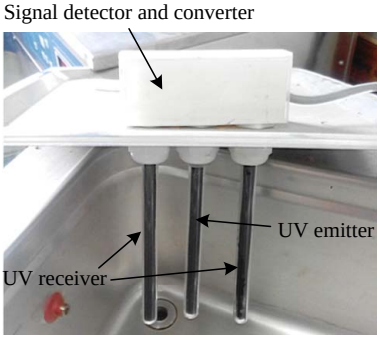

Fig. 2 Photograph of the UV transceiver and signal converter.

In this system, a temperature sensor of type AD590 has been attached to the treatment tank to give the feedback signal for the temperature controller and to monitor the tank temperature. The temperature can be controlled by the temperature controller through presetting a certain temperature value such as 20 °C, 28 °C or 40 °C, etc.

In addition, to obtain the most suitable ultrasonic irradiation, twelve transducers with different resonant frequencies in each working group were evenly distributed on a panel (350×300×2 mm). For improving the precision of optimal frequency as much as possible, the resonance frequency of each transducer was maintained at approximately interval error of 0.67 kHz. Therefore, in the course of determining optimal ultrasonic frequency, even if there is a frequency drift, a certain transducer can work under the best resonant condition.

3 Experimental

3.1 Materials and reagents

Dried tangerine peels were collected from a local market in Bing Hu district, Wuxi, Jiangshu province, China. In our laboratory, the dried tangerine peels were grounded with a blade mixer to obtain 0.45-1 μm particle size. The ground samples were kept in plastic inside desiccators before use. All chemical reagents used in experiments were of analytical grade and purchased from Tianjin Chemical Factory, Tianjin, China.

3.2 Extraction method and analysis

The grounded powders of 5 g were first put into a 3000 m-l beaker 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 beakers were immersed into the ultrasonic bath for irradiation. Finally, extracts were filtered off through 0.45 μm microporous membrane and the filtrate was collected for High performance liquid chromatographic (HPLC) analyses.[18] All experiments were performed in duplicates.

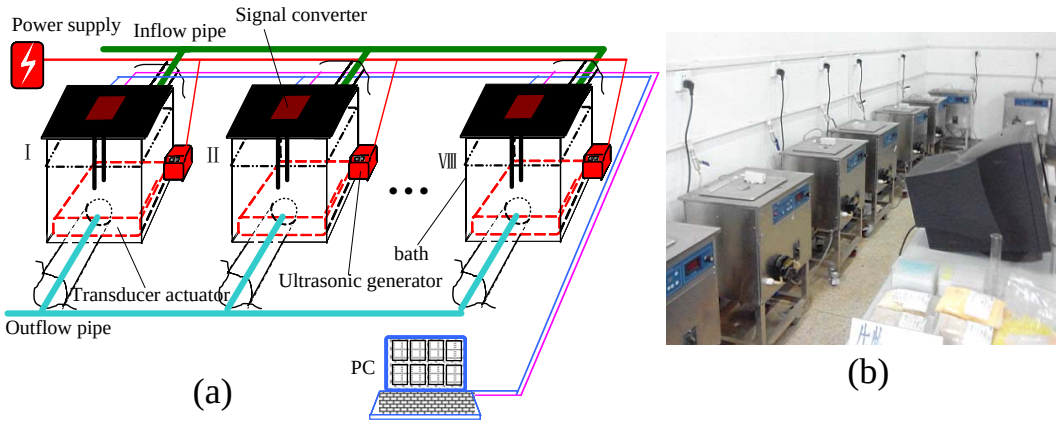

Fig. 1 Experimental setup for the extraction system: (a). Schematic diagram; (b). Photograph.

The dried samples were extracted employing our designed equipment using only methanol as the extracting solvent at room temperature (28 °C). Extraction time was performed from 0 to 60 min under ultrasonic power level of 30 W. The curves of extraction efficiency in experiments can be real-time on-line displayed on the computer by laboratory virtual instrument engineering workbench (labVIEW) software. The samples extracted were collected from the optimal working group and analyzed by HPLC, which are finally used for comparison with the results of extraction.

3.3 Determination procedure of optimal ultrasonic frequency

To obtain accurately the optimal frequency of the ultrasonic extraction from the broad frequency band range, the broad band was evenly divided into eight equal narrow bands with 8 kHz. The flow chart of determination procedure of optimal ultrasonic frequency was shown in the Fig. 3. In this design, the frequency determination procedure involved two steps.

Step 1: All working groups began to determine the optimal ultrasonic frequency band after configured the operating parameters including frequency bands (18-82 kHz), ultrasonic power level (30W), etc. As a certain working group detected the highest efficiency band by UV transceiver, the computer would check automatically to decide if the optimal frequency band is exactly equal to the upper band (74-82 kHz). If not, then started the second step. Otherwise, reset the frequency bands outside the upper band (>82 kHz) and Corresponding transducers, and repeated the above step.

Step 2: In this step, the frequency bandwidth of each working group was decreased from 8 kHz to 1 kHz by reconfiguring relevant transducers. If the optimal frequency band appeared at 42-50 kHz, the frequency bandwidth of each working group will be reset at 42-43 kHz, 43-44 kHz, 44-45 kHz,

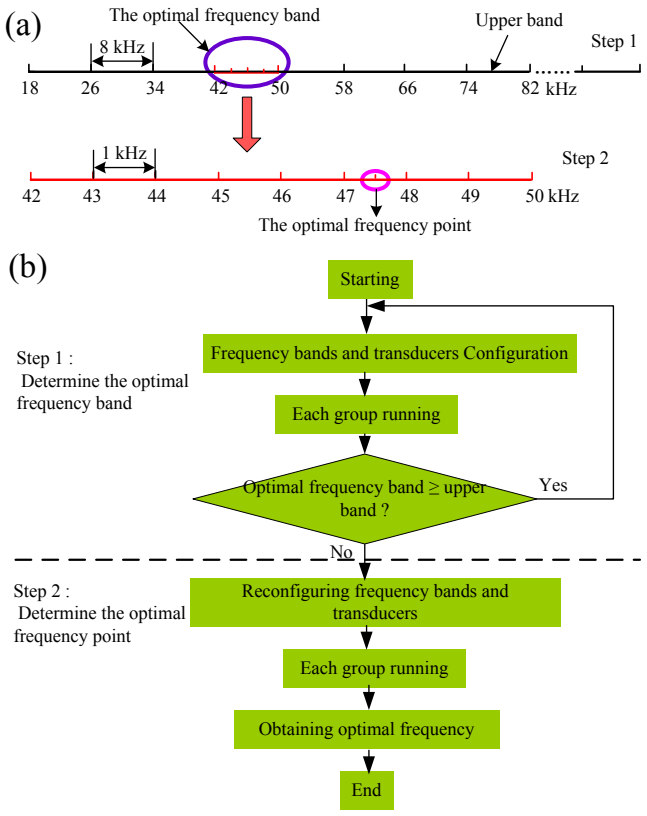

Fig. 3 Determination procedure of optimal ultrasonic frequency: (a). Schematic diagram; (b). Flow chart.

etc. Meanwhile, the transducers of each working groups were replaced, which the center frequency of each frequency band was the same as the corresponding natural frequency of transducer. The extraction experiment was restarted by substituting treatment liquid in every working group. This procedure was carried out similar with the first step. When the highest extraction efficiency appeared in a certain working group, the optimal ultrasonic frequency was just the center frequency of the corresponding band.

4 Results and discussion

4.1 The extraction efficiency of hesperidin from tangerine peels using our system at room temperature (28 °C) and 30W

4.1.1 The graphs of extraction efficiency for determination of optimal frequency band using labVIEW. The study for the extraction efficiency of hesperidin from tangerine peels was performed under different frequency bands (18-26 kHz, 26-34 kHz, 34-42 kHz, 42-50 kHz, 50-58 kHz, 58-66 kHz, 66-74 kHz, 74-82 kHz) by maintaining conditions of other factors, including ultrasonic power of 30W, 60% aqueous methanol, temperature of 28 °C and the ratio of solvent volume to material weight (5:1). The graphs of experimental result are shown in Fig.4. It can be found that the extraction efficiency increased with the rise of extraction time durations for all working groups. The extraction results increased within the initial 0-40 min, then reached the maximum of extraction efficiency. Finally, the rate of change increased slowly until up to zero. It was noted that the 4th working group (42-50 kHz) took only 25 min to reach the maximum value of extraction efficiency, which is less than that of others. In addition, the extraction efficiency of hesperidin from tangerine peels decreased with the deviation degree of different frequency band. In the 8th working group (74-82 kHz), the extraction efficiency reduced to a minimum value. The results might be due to the reasons as following:

In the process of extraction, not all bubbles are capable of producing significant cavitation effects. The greatest coupling of the ultrasonic energy will occur when the natural resonance frequency of the bubble is equivalent to the ultrasonic frequency.[19,20] Considering the surface tension of the viscous medium energy loss and the viscosity of the medium, the natural resonance frequency equation of extraction bubble was deduced by Huang. JL et al.[21]

$$f_r = \frac{1}{2\pi R_e}\sqrt{[\frac{3\gamma}{\rho}(P_0+\frac{2\sigma}{R_e}-\frac{2\sigma}{\rho R_e})]-(\frac{2\eta}{\rho R_e^2})^2} \qquad (1)$$

Where γ is the adiabatic constant, P_0 is the static sound pressure, σ and η are the surface tension and the viscosity of the liquid, respectively, and R_e is the bubble radius at equilibrium. Equation (1) indicated that if ultrasonic frequency was less than the natural resonant frequency (f_r) of the bubble, extraction yield would increase as the ultrasonic frequency increase. On the contrary, extraction yield decreased with increasing ultrasonic frequency. The resonance size of bubbles at given ultrasound frequency was also be estimated by Phillips et al.[22] The ultrasonic frequency of 42-50 kHz in the 4th working group might be closer to the resonance frequency of bubbles, so that it could reach the maximum value of extraction efficiency in a short time.

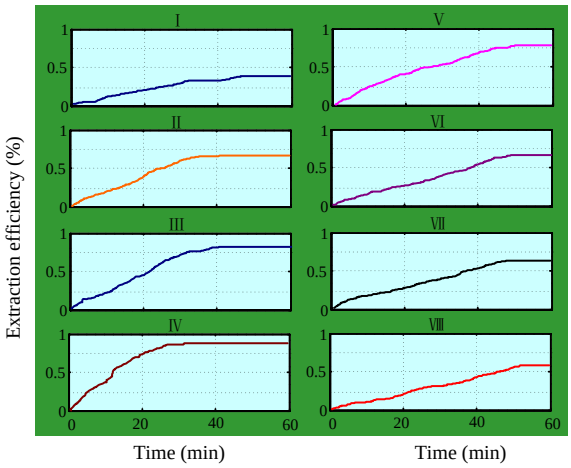

Fig. 4 The graphs of extraction efficiency of hesperidin from tangerine peels using methanol with different frequency bands (18-26 kHz, 26-34 kHz, 34-42 kHz, 42-50 kHz, 50-58 kHz, 58-66 kHz, 66-74 kHz, 74-82 kHz) at room temperature (28 °C) and 30 W.

4.1.2 The extraction efficiency for determination of optimal frequency point. The extraction results carried out for hesperidin from tangerine peels using methanol with different frequency points (42.5 kHz, 43.5 kHz, 44.5 kHz, 45.5 kHz, 46.5 kHz, 47.5 kHz, 48.5 kHz, 49.5 kHz) at room temperature (28 °C) and 30W is represented in Fig.5.

It can be seen that the extraction efficiency of hesperidin from tangerine peels increased gradually as the ultrasonic frequency increase, the frequency point of the highest extraction efficiency appeared at 47.5 kHz. After that, the extraction efficiency declined along with the rise of ultrasonic frequency. It is worth noting that the extraction efficiency between 42 kHz and 50 kHz in this step were higher than that of the first step. Moreover, the maximum value of extraction efficiency at 47.5 kHz is significantly higher than that of others. The result may demonstrate that the cavitation yield drops with increasing in ultrasonic frequency because the cavitation bubbles tend to be smaller and less energetic, resulting in falling the extraction efficiency.[23] Previous study has conformed to the similar trend

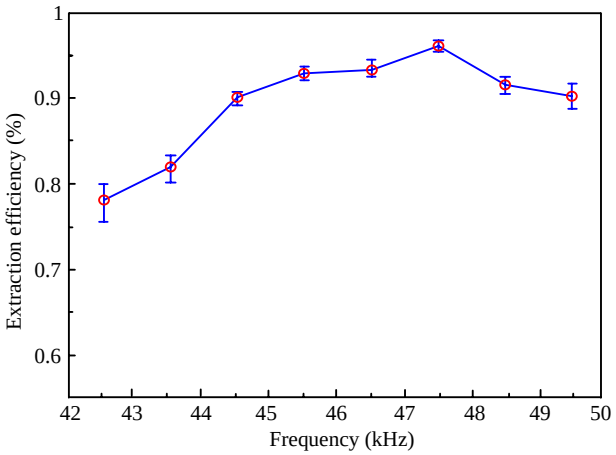

Fig. 5 The extraction efficiency of hesperidin from tangerine peels using methanol with different frequency points (42.5 kHz, 43.5 kHz, 44.5 kHz, 45.5 kHz, 46.5 kHz, 47.5 kHz, 48.5 kHz, 49.5 kHz) at room temperature (28 °C) and 30 W.

that low frequency was found to be preferable.[24] On the other hand, transducers of the 6th working group (47-48 kHz) may all work under a resonance condition, in which every transducer can produce a maximum output power that cause more disintegration of cells and gives higher mass transfer. Owing to a decrease in the amount and intensity of cavitation in liquids at high frequency, in which the rarefaction cycles time for bubbles to grow become shorter.[25]

4.2 Comparison of extraction yields for hesperidin from tangerine peels between our extraction system and existing ultrasonic extraction method

To evaluate the performance of our extraction system, this system was compared with the ultrasound-assisted extraction method proposed by previous study[18] for hesperidin from tangerine peels.

In this study, the existing ultrasound-assisted extraction experiments were carried out in a rectangular ultrasonic bath (KQ-250DE, Kunshan Ultrasound Co. Ltd., China; inner dimension: 300×240×150 mm) with an ultrasound power of 30 W and ultrasonic frequency of 20 kHz, 60 kHz, 100 kHz. The extraction temperature was maintained at 30 °C. The sample beakers were immersed into the ultrasonic cleaning bath for irradiation under fixed extraction variables including methanol, solvent to solid ratio of 5:1, extraction of 60 min. Finally, extracts were filtered off through 0.45 μm membrane filter and the filtrate was collected for HPLC analyses. All samples were prepared and analyzed in triplicate.

ther extraction conditions for our extraction system such as power of 30W, methanol as a solvent, the same particle size and solid-liquid ratio in experiments were examined. Quantitative HPLC analysis was conducted as the procedures described by previous research. Under these conditions, the comparison of extraction yields for hesperidin from tangerine peels was listed in the table 1.

For all cases shown, the yields of hesperidin with existing ultrasonic extraction method under three frequencies all were lower than those with our extraction system (at 47.5 kHz). Moreover, the latter gave 8.9 mg/g greater yields of hesperidin compared with the former at 60 kHz. In addition, the extraction yields using existing extraction method reached a peak value for 60 min while those of our extraction system was only for 25 min, even the former work at a temperature of 40 °C, but our extraction system is only at room temperature (28 °C). The results indicated the maximum yield of extraction appeared at 47.5 kHz rather than 60 kHz, which might be attributed to the fact that the optimal ultrasonic frequency could cause bubble collapse by driving the bubble into resonance. In fact, the extraction yield depends on the degree of cavitation activity. When the ultrasonic frequency is closer to the natural resonance frequency of bubbles, the bubbles collapse violently, which may be favorable to enhance extraction yield. As a result, 47.5 kHz was just the optimal ultrasonic frequency of hesperidin from tangerine peels, which could gain maximum yield of hesperidin.

Fig.6 also showed a comparison of extraction yields for hesperidin between our extraction system and existing extraction method under the same extraction conditions except the ultrasonic frequencies. Here, the ultrasonic frequency of our extraction system was set at 47.5 kHz. It could be seen that the extraction yield of our extraction system was more than those of existing extraction method. Moreover, our extraction system took only 25 minutes to reach the maximum of extraction yield while existing extraction method lasted more than 60 minutes.

It suggested that ultrasonic extraction can attract cells swelling and enlarge the pores of the cell wall. Sound swelling can improve the rate of mass transfer, and lead to the increased extraction efficiency and reduced extraction time duration.[26–28] In addition, shorten extraction time is probably linked to the fact that the ultrasonic frequency of 47.5 kHz might be more closer to the resonant frequency of the bubbles. Moreover, suitable frequency can cause more disintegration of cells and give higher mass transfer which results in more extraction yields. Therefore, it might be conclude that our extraction system could significantly enhance the extraction yield. Moreover, it could also efficiently lower the working temperature and shorten the extraction time, comparing with existing ultrasound extraction method.

Table 1 Extraction yields for hesperidin from tangerine peels by existing ultrasonic extraction method compared to that of our extraction system

Methods	Power (W)	Time (min)	Temperature (°C)	Frequency (kHz)	Extraction yield (mg/g)
Existing extraction method	30	60	40	20	44.6
Existing extraction method	30	60	40	60	56.4
Existing extraction method	30	60	40	100	49.2
Our extraction system	30	25	28	47.5	65.3

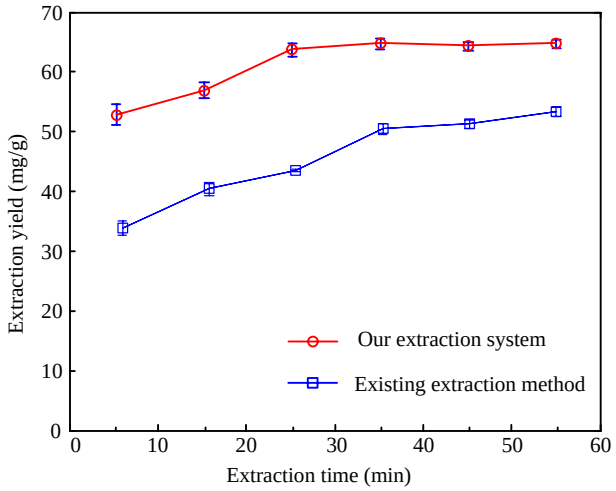

Fig. 6 Comparison of extraction yields for hesperidin from tangerine peels between our novel extraction system and existing ultrasonic extraction method under the same extraction conditions.

5 Conclusions

This paper proposed an ultrasonic extraction system that could determine the optimal frequency for various plant materials in a wide band range. The extraction results could be on-line displayed on a computer, which could shorten experimental time and reduce operation cost. A determination method for optimal ultrasonic frequency was carried out by two steps including determining optimal frequency band and frequency point, respectively. An UV transceiver with a pair of ultraviolet emitter and receiver on each working group was used to achieve precision detection for different analytes. A comparative experiment of hesperidin from tangerine peels was also performed under the same extraction conditions except the ultrasonic frequencies. The results from this study showed that the maximum of extraction yield appeared at 47.5 kHz. It indicted that this system could significantly improve the extraction yield by determining optimal ultrasonic frequency. Moreover, the frequency range of this system could be extended according to different plant materials by replacing transducers in each working group.

Acknowledgements

The present work was supported by the National Natural Science Foundation of China (309716899), and the Programme of Introducing Talents of Discipline to Universities (B12018).

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