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Ultrasound-assisted extraction of ginseng saponins from ginseng roots and cultured ginseng cells

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Abstract

Ultrasound-assisted extraction was evaluated as a simpler and more effective alternative to conventional extraction methods for the isolation of ginsenosides (saponins) from various types of ginseng. The ginseng samples were extracted with different solvents, under either direct sonication by an ultrasound probe horn or indirect sonication in an ultrasound cleaning bath. The ultrasonic extraction was compared with the conventional method of refluxing boiling solvents in a soxhlet extractor, on the yields of both the total saponin isolated by thin-layer chromatography and the individual ginsenosides by high performance liquid chromatography. It was found that the sonication-assisted extraction of ginseng saponins was about three times faster than the traditional extraction method. The ultrasonic extraction was not only more efficient but also convenient for the recovery and purification of the active ingredients of plant materials. In addition, the sonication-assisted extraction can be carried out at lower temperatures which are favorable for the thermally unstable compounds.

Keywords: Extraction; Ginseng; Ginsenoside (saponins); High performance liquid chromatograph; Thin layer chromatograph; Ultrasound

1. Introduction

Extraction is the first important step for the recovery and purification of active ingredients of plant materials. The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents and the use of heat and/or agitation to increase the solubility of materials and the rate of mass transfer. Usually, the traditional techniques require long extraction hours and have low efficiency. Moreover, many natural products are thermally unstable and may degrade during thermal extraction. Recently there have been numerous reports on the application of high intensity or power ultrasound in the extraction of various phytochemicals, such as alkaloids, flavonoids, polysaccharides, proteins and essential oils, from various parts of plant and plant seeds [1–7]. The extraction of organic compounds from various plant materials can be significantly improved with the aid of intense ultrasound, achieving higher product yields at reduced processing time and solvent consumption. In addition, ultrasonic extraction can be carried out at lower temperatures, avoiding thermal damage to extracts and loss of volatile components in boiling. It has been suggested that the improvement of solvent extraction from plant material by ultrasound is due mainly to the mechanical effects of acoustic cavitation, which enhances both solvent penetration into the plant material and the intracellular product release by disrupting the cell walls [8].

The objectives of this work were to evaluate the sonication-assisted solvent extraction of ginsenosides (triterpene saponins) from ginseng roots and to establish an ultrasonic extraction protocol for isolation of the total saponin and the major ginsenosides in ginseng roots. Ginseng is a common name for various *Panax* plants, particularly *Panax ginseng* (Korea and Chinese ginseng) and *P. quinquefolium* (American ginseng). They are among the most precious and famous plant herbs, which, mainly their roots, are widely used for health foods and traditional medicine. Ginsenosides are known as the principal ingredients of ginseng [9]. Although ultrasound has been frequently used in the analysis of ginseng saponins [10,11], a more full evaluation of the sonication-assisted extraction is still needed to establish a general protocol. In this study, sonication-assisted extraction was tested for various ginseng roots with

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different extracting solvents. The ultrasonic extraction was compared with the classical extraction method of refluxing boiling solvents in a soxhlet extractor.

2. Experimental

2.1. Plant materials and extracting solvents

Four types of ginseng roots were tested in this study, the American ginseng root (*P. quinquefolium*), fresh Chinese ginseng root (*P. ginseng*) and red Korean ginseng root (*P. ginseng*) and *P. ginseng* root cells from suspension culture. The ginseng roots were purchased from local drug stores and the ginseng (root) cells were collected from suspension cultures maintained in our own lab with the conditions as described elsewhere [12]. All the ginseng samples were dried and pulverized to less than 300-mesh size before the extraction. Three different solvents were used for the extraction of ginseng saponins from the plant materials, i.e., pure methanol, water–saturated *n*-butanol and water with 10% methanol (vol.%).

2.2. Extraction of ginseng saponins

The conventional extraction method of boiling and refluxing the solvent in a soxhlet extractor was used as a control for comparison with the ultrasound-assisted extraction methods. A 5-ml soxhlet extractor was used, with which each 200 mg of a ginseng sample was extracted with 15 ml of a solvent. The solvent was boiled and refluxed in the extractor for a period of 1, 2 or 8 h.

In sonication-assisted extraction, each 200 mg of a ginseng sample was mixed with 15 ml of extracting solvent in a 50 ml conical polypropylene centrifuge tube of 3.0 cm in diameter. For indirect sonication, the sample tube was immersed in an ultrasound cleaning bath; for direct sonication, a sonicator probe horn was fitted into the sample tube with its tip dipped into the solvent. The ultrasound cleaning bath was a CREST 1875 (Crest Ultrasonics, Trenton, NJ, USA), which has a frequency of 38.5 kHz and a maximum peak power of 810 W. The power level was set at the maximum (level 9) and the bath temperature at 25°C during the extraction. The sample tubes in the bath were shaken continuously with an orbital shaker at 100 rpm and the liquid level inside the tube was about 1.0 cm below the liquid surface in the bath. The sonicator probe horn (with a 3-mm diameter tip) was connected to a 600-W Cole–Palmer ultrasound microprocessor (Cole–Palmer, Vernon Hills, IL, USA) having a frequency of 20 kHz, which was operated at no pulse and 22% amplitude. The sample tube was immersed in ice during the extraction. The tip was dipped about half way into the 15 ml extracting liquid in the 50 ml centrifuge tube, which was found to produce the maximum extraction rate (based on preliminary test of three different tip positions).

The maximum temperature in the sample tubes during the 1–2 h extraction period was stabilized at 25–27°C with the probe horn, and 38–39°C in the cleaning bath. The temperature in the sample tubes was measured with a type-K thermocouple digital thermometer (Cole–Palmer, Vernon Hills, IL). The ultrasound power actually delivered to the extracting liquid by the sonic bath and probe horn was determined by the calorimetric method similar to that used in Ref. [13]. At the machine settings used for the extraction, the actual power delivered to each sample tube was found to be 8.2 W by the probe horn (at 22% amplitude) and 3.5 W by the sonic bath (at level 9), respectively.

There was no solvent renewal throughout the period of extraction in any of the above extraction processes. After the extraction, the sample tubes were centrifuged at 2000 rpm for 5 min, and then 10 ml of the liquid extract was collected and evaporated to dryness under vacuum at 40°C. The residue was dissolved in 0.5 ml acetonitrile to be used for the following thin layer chromatographic (TLC) and the high performance liquid chromatographic (HPLC) analysis of the saponins. With the ginseng cell extract, however, an additional step of purification was performed after the evaporation because of the high impurity contents. The residue from the above evaporation was dissolved in 2 ml of water and applied to a Sep-Pak C18 cartridge (Waters, Milford, MA, USA). The column was washed with water (10 ml) and 10% methanol (5 ml), and then eluted with pure methanol (5 ml). The methanol eluate containing the saponins was evaporated and the residue was dissolved in acetonitrile.

For each sample, there were three to four replicates from the very beginning of the extraction to the final quantification of saponins. Each data point reported in the results is the mean of these replicate measurements.

2.3. Determination of the total saponin

The measurement of total saponin followed the colorimetric method described in Ref. [14] with modifications. The method was based on a color reaction of the acid-hydrolysis products of the saponins, sapogenins, with vanillin. The purified ginseng extract solution in acetonitrile (10 μl) was applied to a TLC plate (Silica Gel 60, UV254, 0.25 mm layer) with chloroform–methanol–water at the ratio of 15:12:2 as the mobile phase. The total saponin spot was located with a ginsenoside standard, then scratched off and mixed with 0.2 ml of acetic acid containing 5% (wt.%) vanillin and 0.8 ml of perchloric acid at 60°C for 15 min. The concentration of total saponin was determined with a spectrophotometer at 560 nm against a calibration curve established with a panaxtriol standard.

2.4. High performance liquid chromatographic analysis of individual ginsenosides

The HPLC conditions were based on Ref. [15] which gave satisfactory resolution of the five major ginsenosides, Rb1, Rb2, Rc, Rd, Rf. The HPLC system consisted of a Waters 515 pump and an Econosphere ODS-2 column of 250 × 4.6 mm dimension and 5 μm packing (Alltech, Deerfield, IL, USA). The mobile phase was acetonitrile–water (30:70) at a flow rate of 1.5 ml/min (isocratic elution). For the analysis, 10 μl of the saponin–acetonitrile solution obtained from the above extraction step was injected into the system. The saponin peaks were detected by a UV detector at 203 nm, and quantified based on calibration curves of ginsenoside standards (Extrasynthese Cedex, France).

2.5. Thin layer chromatographic identification of individual ginsenosides

The same TLC plate as aforementioned was used while the mobile phase was replaced by butanol–ethyl acetate–water (4:1:5). The ginsenosides were visualized as brown spots by spraying H_2SO_4–H_2O (1:1) and heating at 105°C in an oven.

3. Results and discussion

3.1. Extraction yield and rate of total saponin

The ginseng samples were first extracted in the two types of sonicator for different periods of time in order to determine the contact time required to achieve the maximum yield of total saponin. Fig. 1 shows the typical trends of saponin yield against sonication time period for the American and Chinese ginseng roots. The extraction yields increased significantly with the sonication period extended from 15 to 45 min for both types of ginseng root and in both sonicators, but increased slightly or leveled off from 60 to 120 min. The results suggest that the ultrasonic extraction period for achieving maximum yield of saponin from the ginseng roots is about 2 h.

Table 1 shows the yields of total saponin from the four types of ginseng root obtained with different extraction methods and extracting solvents for various periods of time. For all the cases shown, the saponin yields of 1–2 h extraction under direct and indirect sonication were all significantly (50–100%) higher than those with the traditional method in the soxhlet extractor. The yields of 2 h ultrasound-assisted extraction were comparable to those achieved by 8 h extraction with the conventional method. This means that the extraction rate of the ultrasound-assisted processes was about three times faster than that of the conventional method. With the sonication-assisted extraction, the saponin yields only showed a small increase with the extraction time increased from 1 to 2 h, suggesting that the extraction had almost reached the maximum or equilibrium yields.

3.2. Extraction yields and rates of individual saponins

The improvement of extraction efficiency by sonication was also confirmed by HPLC analysis of the individual ginsenosides of the ginseng samples. Table 2 is a summary of the HPLC results of the five major ginsenosides and Fig. 2 compares the sums of the five ginsenosides extracted from the Chinese ginseng root by different extraction methods and solvents. For 1–2 h extraction, both of the ultrasonically assisted methods produced higher yields of individual saponins than the conventional method. The yields of 2 h ultrasonic extraction were even higher than those extracted for 8 h by the traditional method. Therefore, the extraction rate under the influence of ultrasound was increased by about threefold compared to the extraction with the soxhlet extractor. Among the five ginsenoside species,

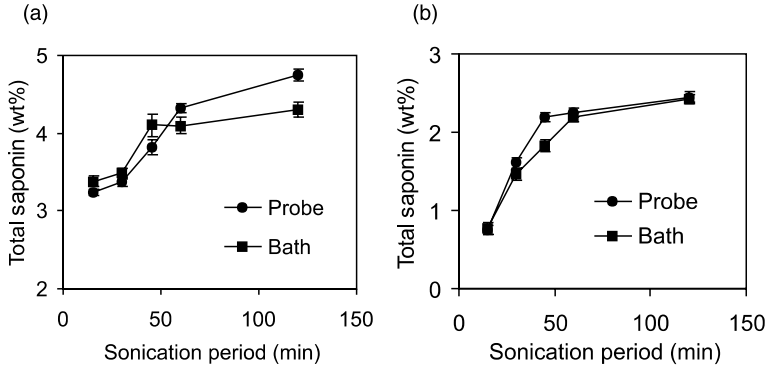

Fig. 1. The saponin yields of sonication-assisted extraction for various periods of time with water–saturated *n*-butanol as the extracting solvent: (a) American ginseng root, (b) Chinese ginseng root.

Table 1
The yields (wt.%) of total saponin extracted from various ginseng samples by different extraction methods[a]

Extracting solvent	Extraction method/time						
	Ultrasound bath		Ultrasonic probe		Soxhlet extractor		
	1 h	2 h	1 h	2 h	1 h	2 h	8 h
American ginseng							
MeOH	3.95	4.00	4.25	4.58	2.00	2.75	4.40
Water–saturated BuOH	4.10	4.30	4.32	4.75	2.10	2.95	4.61
Water with 10% MeOH	4.00	4.20	4.20	4.65	2.10	2.90	4.52
Chinese ginseng							
MeOH	2.10	2.30	2.15	2.31	1.20	1.58	2.28
Water–saturated BuOH	2.20	2.42	2.25	2.45	1.22	1.62	2.46
Water with 10% MeOH	2.15	2.38	2.20	2.40	1.15	1.70	2.35
Korean red ginseng							
MeOH	2.95	3.20	2.90	3.22	1.68	2.25	3.30
Water–saturated BuOH	3.00	3.35	3.12	3.45	1.75	2.60	3.30
Water with 10% MeOH	3.13	3.25	3.20	3.30	1.60	2.00	3.25
Ginseng cells							
MeOH	2.00	2.11	2.05	2.20	1.35	1.45	2.00
Water–saturated BuOH	2.10	2.20	2.15	2.30	1.45	1.65	2.10
Water with 10% MeOH	2.10	2.20	2.10	2.22	1.40	1.50	2.15

[Note: MeOH = methanol, BuOH = *n*-butanol.]
[a] Each point is the mean of three to four replicates with a standard deviation no more than 10% of the mean.

Table 2
The yields (wt.%) of five major ginsenosides extracted from Chinese ginseng root by different extraction methods

	Extraction method/time						
	Ultrasound bath		Ultrasonic probe		Soxhlet extractor		
	1 h	2 h	1 h	2 h	1 h	2 h	8 h
Extracting solvent: MeOH							
Rb1	0.273	0.301	0.220	0.291	0.137	0.175	0.267
Rb2	0.101	0.112	0.102	0.113	0.060	0.090	0.115
Rc	0.196	0.250	0.175	0.246	0.104	0.166	0.233
Rd	0.046	0.085	0.051	0.080	0.025	0.032	0.050
Rf	0.095	0.120	0.102	0.135	0.052	0.098	0.127
Water–saturated BuOH							
Rb1	0.304	0.342	0.265	0.312	0.150	0.195	0.278
Rb2	0.115	0.131	0.110	0.127	0.069	0.080	0.122
Rc	0.253	0.290	0.252	0.281	0.111	0.175	0.260
Rd	0.101	0.118	0.102	0.109	0.050	0.071	0.090
Rf	0.135	0.152	0.145	0.168	0.068	0.101	0.125
Water with 10% MeOH							
Rb1	0.302	0.343	0.285	0.331	0.181	0.235	0.312
Rb2	0.090	0.098	0.075	0.105	0.041	0.052	0.085
Rc	0.214	0.234	0.201	0.222	0.104	0.126	0.235
Rd	0.082	0.099	0.075	0.075	0.020	0.031	0.048
Rf	0.091	0.102	0.083	0.098	0.048	0.057	0.110

Rb1 and Rd were most significantly stimulated by the sonication in their release from the ginseng powder.

Significant enhancing effects of sonication were also observed on the extraction of other ginseng samples (data not shown). Fig. 3, for example, shows the chromatograms of four major ginsenosides in the American ginseng extracted with water–saturated *n*-butanol for 2 h under sonication and 8 h in the soxhlet extractor without sonication.

3.3. Effect of extracting solvents on the yields and analysis of saponins

For most of the ginseng samples, the highest yields of total and individual saponins were gained with water–saturated *n*-butanol (Tables 1 and 2). The difference was more obvious for the individual ginsenosides obtained from the HPLC analysis (Fig. 2). In addition, the ginsenosides extracted by *n*-butanol also seems to have a

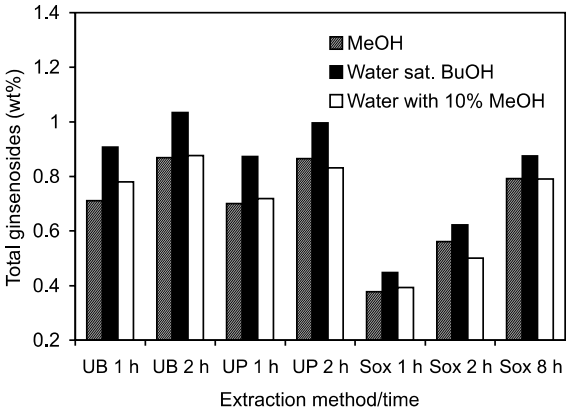

Fig. 2. The sums of five major ginsenosides detected by HPLC of the Chinese ginseng root, extracted with different solvents in: UB – ultrasound cleaning bath, UP – ultrasound probe horn, and Sox – soxhlet extractor.

better resolution in HPLC, such as the chromatograms of Chinese ginseng extracted in the ultrasonic bath for 1 h (Fig. 4).

Although the use of *n*-butanol as the extracting solvent produced higher yields of ginsenosides than methanol, its disadvantage over methanol is a higher boiling point (118°C, versus 65°C for MeOH), which makes it more difficult to evaporate. When it is used as the extracting solvent in the conventional thermal method, the higher boiling temperature may cause thermal decomposition of some ginsenosides. Some ginsenosides, especially the malonyl ginsenosides, are thermally unstable which may degrade at high temperatures [16]. The heat sensitivity of some saponin species of ginseng was also detected by TLC of ginseng extracts in our experiment. As shown by Fig. 5, the two lower spots shown for the extracts obtained from 2 h extraction with water–saturated *n*-butanol by all the three methods disappeared after 8 h thermal extraction in the soxhlet extractor. Meanwhile, the extract of 8 h thermal extraction had two new spots on the top front. The result again suggests the advantage of ultrasound-assisted extraction, which can be achieved at lower temperatures, for the recovery of temperature-sensitive ingredients of natural products.

3.4. Comparison of the sonication probe with the sonic bath for the extraction

In comparison of the two sonication methods employed in this study, direct sonication by the probe horn could provide much higher ultrasound energy to the samples than indirect sonication by the cleaning bath (8.2 versus 3.5 W). In view of the total saponin concentrations (Table 1) obtained with the two instruments, however, the probe horn did not show a clear advantage over the sonic bath for the extraction. As a matter of fact, the extraction rate with the cleaning bath was even slightly higher than that with the probe horn based on the total amounts of the major ginsenosides shown in Table 2. The higher rates of saponin release in the ultrasound cleaning bath may be partially attributed to the agitation and the higher temperature (38–39°C) in the sample tubes with the sonic bath than that with the sonicator probe horn (25–27°C). For analytical purpose which requires to process many small-volume samples, an ultrasound bath may be more desirable than the probe horn for the extraction. First of all, the bath can process many samples at one time while the probe horn only allows for one at a time. Secondly, sonication with the cleaning bath is non-intrusive to the sample which will eliminate the possible contamination and loss of the extract. Moreover, the cleaning bath is usually much quieter than the probe horn during the operation. Therefore, an ultrasonic cleaning bath may be more convenient and efficient for the extraction of large number of small-volume samples. In addition, the renewal of solvent one or two times during the extraction

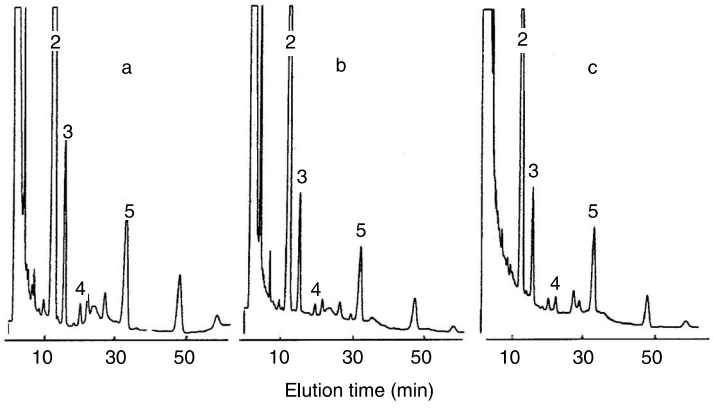

Fig. 3. HPLC of ginsenosides (2 – Rb1, 3 – Rc, 4 – Rb2, 5 – Rd) of American ginseng extracted with water–saturated *n*-butanol: (a) in ultrasound bath for 2 h, (b) under ultrasonic probe horn for 2 h, and (c) in soxhlet extractor for 8 h.

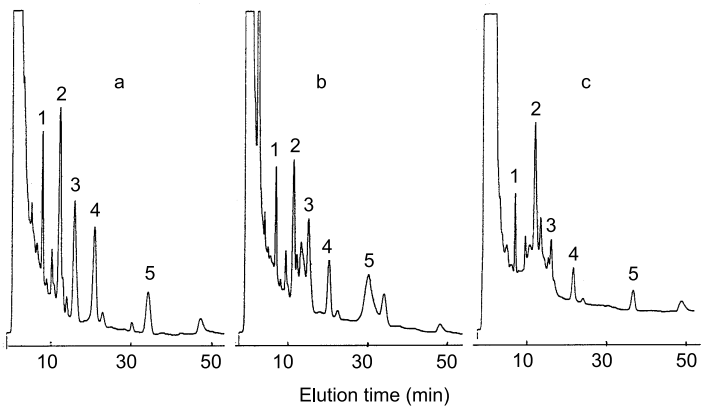

Fig. 4. HPLC of ginsenosides of Chinese ginseng extracted in ultrasound cleaning bath for 1 h with (1 – Rf, 2 – Rb1, 3 – Rc, 4 – Rb2, 5 – Rd): (a) water–saturated *n*-butanol, (b) methanol, and (c) water with 10% methanol.

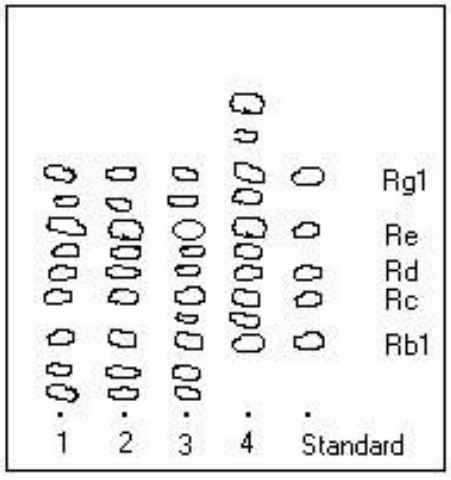

Fig. 5. TLC of ginsenosides of American ginseng extracted with water–saturated *n*-butanol: (1) in ultrasound cleaning bath for 2 h, (2) under ultrasonic probe horn for 2 h, (3) in soxhlet extractor for 2 h, and (4) in soxhlet extractor for 8 h.

may be necessary for thorough extraction. For large-scale applications, however, direct sonication using the probe horn combined with mechanical agitation may be more efficient [3].

4. Conclusions

Ultrasound has proven to be a much simpler and more effective means than the traditional extraction method of refluxing boiling solvents for the extraction of ginseng saponins from various ginseng roots. The ultrasound-assisted extraction of saponins from the ginseng roots was three times faster than the conventional method of thermal extraction. In addition, ultrasound-assisted extraction can be carried out at lower temperatures which can avoid the degradation of thermally unstable ingredients in plant materials. With all these merits, ultrasound-assisted extraction should be considered for wider application in the isolation and purification of phytochemicals from plants.

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