Physicochemical and in vitro digestion characteristics of size-different red ginseng powders

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Abstract The aims of the present study were to prepare different-sized red ginseng powders and investigate the particle size effect on the release property of ginsenosides in in vitro digestion conditions. Ultrafine powder showed bimodal particle size distribution with a large peak at around 100 μm and small peak at around 10 μm , differently from fine powder showing unimodal distribution at 100 μm . The specific surface areas of fine- and ultrafine powders were 48.72 ± 6.41 and $86.74 \pm 5.96 \text{ m}^2/\text{g}$, respectively. Time-dependent release property of the powders in the simulated gastrointestinal fluids was determined by quantifying ginsenoside Rg1 released. The initial and final concentrations of ginsenoside Rg1 released was higher in ultrafine powder than fine one. It is expected that particle size reduction and corresponding increase in the specific surface area have a potential to improve the release of ginsenosides in the gastrointestinal tract and enhance the chances to be absorbed in human body.

Keywords Red ginseng · Ultrafine powder · Particle size effect · In vitro digestion conditions · Release property

Introduction

Panax ginseng has traditionally been a widespread herbal medicine and served as a major prescription in oriental countries for more than 2000 years [1]. Today, it still occupies a predominant position in global herbal markets as an extract and powdered form. Ginsenosides or ginseng saponins are unique and principal bioactive compounds in ginseng roots and more than forty different forms of ginsenosides have been identified [1]. General therapeutic and pharmacological effects of ginsenosides have been extensively evaluated and the effects includes vasorelaxation, antioxidation, anti-stress, anti-inflammation, anti-viral, anti-cancer, anti-fatigue, stimulating immune functions and maintaining homeostasis [2, 3]. Red ginseng is a processed product of *Panax ginseng* by steaming and drying the fresh ginseng. Many new derivatives of ginsenosides appear in red ginseng, differently from raw or white ginseng. Furthermore, the contents of minerals, vitamins, polyphenols, functional amino acids and essential oils increase. Thus, red ginseng has different pharmacological activities compared with white ginseng resulting from structural and chemical transformations of constituents during heat treatment [4].

Red ginseng products have generally been launched in the markets as an extract rather than powdered form since the extract form is known to be more bioavailable than powdered form. However, lots of hydrophobic and/or amphiphilic bioactive compounds are hardly extracted on a hot water extraction. Powdered form, on the other hand, contains all bioactive compounds regardless of hydrophilic-hydrophobic characteristics of the compounds [5]. Low solubility and bioavailability have been major limitation of widespread applications of powdered herbal plants in food products. Recently, noble grinding

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technologies have been developed to prepare ultra- and superfine powders with noticeable properties such as high solubility and dispersibility, modified physicochemical properties, enhanced release and gastrointestinal absorption, and improved biological functions [6–8]. Zhong et al. [6] reported that the release of polyphenols and flavonoids from pomegranate peel powders in SGF (simulated gastric fluid) increases as particle size decreases. In another report, Zhao et al. [9] demonstrated enhanced release rate of a bioactive compound, dracorhodin, from superfine Qili powder compared to fine one in the aqueous solution. Moreover, Lee et al. [10] demonstrated the particle size effect on extraction yield of bioactive compounds of red ginseng and improved inhibitory effects on LPS-induced cytokine. There have been lots of in vitro and in vivo studies evaluating biological and pharmacological benefits of medicinal herb powders, and particle size effects are clearly evident [11]. However, in vitro release properties of bioactive compounds from powdered medicinal herb and subsequent particle size effects have merely been investigated.

Therefore, the objectives of the present study were to prepare red ginseng powders of different particle size distributions and investigate their effects on the release properties of bioactive compounds in the simulated gastrointestinal conditions. For this purpose, red ginseng powders of different particle size (fine- and ultrafine particle) were prepared and their physicochemical properties such as particle size distribution, morphology, specific surface area, zeta-potential and vapor sorption properties were evaluated. In vitro release properties were evaluated by comparing HPLC chromatograms of ginsenoside Rg1 released from ginseng powders as a function of time.

Materials and methods

Preparation of fine- and ultrafine red ginseng powders

Red ginseng roots (6 year-old) purchased from a local market (Seoul, Korea) was sliced into chips with approximate 10 mm length. The red ginseng chips were milled coarsely using a pin mill then furtherly milled using an air classifying mill (ACM-250, Hankook Crusher Co. Ltd., Incheon, Korea). The air classifying mill pulverized the pin-milled red ginseng powder until a target particle size distribution was achieved. When the particle size of red ginseng powder reduced enough to pass through a centrifugal air-classification system, the particles were transported to an outlet; otherwise the particles remained in the grinding zone and pulverized furtherly. Thereafter, the red ginseng powder was passed through the sieves in stacks

(#70 with 200 μm aperture, #170 with 100 μm aperture, and #270 with 50 μm aperture) to obtain the powder with less than 50 μm particle size fraction (fine red ginseng powder). In order to prepare ultrafine powder, 30 g of the fine red ginseng powder was pulverized furtherly using a ball mill (BML-2, Daihan Scientific Co. Ltd., Seoul, Korea) with 100 zirconia balls (3 mm in diameter). The ball mill was operated at 100 rpm of the milling speed for 12 h and subsequently ultrafine red ginseng powder was produced.

Scanning electron microscopy (SEM)

The particle shape and size of fine- and ultrafine red ginseng powders were observed using a field-emission scanning electron microscopy (FE-SEM, S-4300, Hitachi, Tokyo, Japan) to verify size. The SEM was operated an accelerating voltage of 4.0 kV for each samples.

Particle size distribution measurement

For the particle size measurement, 0.1 g of red ginseng powder was dispersed in 100 mL of ethanol with 0.1 g Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) and stirring at 25 °C on a magnetic stirrer (Wisestir MS-MP4, Daihan Scientific Co. Ltd.) at 300 rpm for 1 h. The red ginseng powder suspension was loaded in a flow cell and its particle size distribution was determined using a particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd., Malvern, Worcestershire, UK) at 25 °C. All samples were measured at least 3 times.

Zeta-potential measurement

To measure zeta-potentials of fine- and ultrafine red ginseng powders, 0.1 g of red ginseng powder was dispersed into 100 mL of deionized water followed that the pH was adjusted to 2, 4, 6, 8, 10, and 12 with 0.1 N HCl or NaOH. One mL of the samples at different pHs was injected in a flow cell of a zeta-potential analyzer (Delsa Nano C, Beckman Coulter, Inc., Fullerton, CA, USA). The zeta-potential of the sample was measured at 5 positions (0.7, 0.35, 0, – 0.35, and – 0.7) of the flow cell. All samples were measured 3 times at 25 °C.

Vapor sorption isotherm and specific surface area measurement

The isotherms of fine- and ultrafine red ginseng powders were measured using a dynamic vapor sorption method. Ten mg of red ginseng powder was uniformly placed onto a sample pan of DVS (DVS intrinsic, Surface Measurement System, London, UK) and isotherm data were obtained on

a adsorption–desorption cycle of vapor. The relative humidity (RH) in the chamber of DVS increased from 0 to 90% and decreased from 90 to 0% at fixed temperature of 25 °C. The Brunauer–Emmett–Teller (BET) specific surface areas of the fine- and ultrafine red ginseng powders were calculated based on the desorption plot obtained in the RH ranges from 5 to 40%.

In vitro digestion test

Release property of fine- and ultrafine red ginseng powders were investigated in the simulated gastrointestinal fluids. For the simulated stomach condition, 10 mL of pepsin solution (30 mg/mL in 0.1 M HCl) was added in 100 mL of 20 mM phosphate buffer in a erlenmeyer flask in which 6 g of red ginseng powder dispersed. Thereafter, the pH of the simulated gastric fluid was adjusted to 2.0 and the flask was incubated in a shaking water bath (Han Yang Scientific Equipment Co., Ltd, Seoul, Korea) at 37 °C and 95 shakes per min for 1 h. For the simulated small intestine condition, the pH of the flask incubated in the simulated gastric fluid was raised to 5.3 with 100 mM NaHCO₃. Afterward, 50 mL of a mixture of lipase, bile extract, and pancreatin (50 mL containing 0.2 mg/mL lipase, 2.4 mg/mL bile extract and 0.4 mg/mL pancreatin in 100 mM NaHCO₃ solution) was added to the flask. Then, the pH of the flask was adjusted to 7.0 with 0.1 N NaOH, and incubated in the shaking water bath at 37 °C and 95 shakes per min for 2 h.

Contents of ginsenoside Rg1 as an index material for the release property of the red ginseng powder in the simulated gastrointestinal fluids were analyzed using HPLC (1200 Series, Agilent Technologies, Inc., Toulouse, France). A μ -Bondapak C18 column (3.9 mm $\times$ 300 mm, 10 μ m particle size, Waters Corp., Milford, MA, USA) was used for all separations. The gradient elution system consisted of water (A) and acetonitrile (B). Separation was achieved by using 70 (A): 30 (B). The column temperature was set at 25 °C. The flow rate was 1.0 mL/min and the injection volume was 10 μ L. The UV detection wavelength was set at 203 nm.

Results and discussion

Morphology and particle size distribution

Morphological differences between the fine- and ultrafine red ginseng powders were evaluated by SEM and the results are shown in Fig. 1. It was observed that the ultrafine red ginseng powders ground by ball milling contained a relatively larger number of smaller particles than the fine one. The ultrafine red ginseng particles ranged in size from $\sim$ 0.1 to 3 μ m while the fine powder contained

larger particles ($\sim$ 10 μ m). Both the fine- and ultrafine red ginseng particles showed various irregular shapes, which are characteristic of the appearance of pulverized herbal plant particles [5]. Several smaller fragments and debris were evident on the SEM micrograph of ultrafine red ginseng powder because of the air classifying milling and subsequent ball milling during preparation of the red ginseng powders.

Particle size distributions of the fine- and ultrafine red ginseng powder are shown as volume percentage versus particle size in Fig. 2. Compared with the unimodal size distribution of fine red ginseng powder with a peak at around 100 μ m, ultrafine powder represented bimodal size distribution with a small peak at around 10 μ m and a large peak at around 100 μ m. The bimodal size distribution of ultrafine red ginseng powder results from the breakup of larger particles by ball milling that followed air classifying milling. The average particle sizes of the fine- and ultrafine red ginseng powders were 109.8 and 87.0 μ m, respectively. There is a shift of mean particle size towards smaller size and the appearance of a bimodal distribution in the ultrafine red ginseng powder.

Zeta-potentials

pH-dependent zeta-potential profiles of an aqueous suspension of fine- and ultrafine red ginseng powders are shown in Fig. 3. At pH 2, the zeta-potentials of fine and ultrafine red ginseng powder suspensions were -0.66 ± 0.25 and 0.77 ± 0.13 mV, respectively. Red ginseng powders rapidly agglomerated or coagulated at pH 2 because they were unstable in the polar aqueous media. As the pH increased, the zeta-potentials gradually decreased towards negative charge up to approximate -20 mV owing to selective adsorption of OH[−] liberated from the excess NaOH added. The red ginseng powders precipitated incipiently even at neutral and high pH since the zeta-potentials were still close to zero. Usually, the particles with more than ± 30 mV of zeta-potential values show moderate dispersing stability in the media. Here, there were no different trends between the pH-dependent zeta-potentials of fine- and ultrafine red ginseng powders. The particles in both fine- and ultrafine red ginseng powder suspensions agglomerated together and subsequently settled down regardless of the particle size. The settling velocity was relatively slow in the ultrafine red ginseng powder suspension.

DVS isotherm and specific surface area

DVS isotherms of the fine- and ultrafine red ginseng powders are shown in Fig. 4, which plots mass change (%) versus relative humidity (RH, %). Adsorption isotherm

Fig. 1 SEM micrographs of (A) fine- and (B) ultrafine red ginseng powders

Fig. 2 Particle size distributions of (A) fine- and (B) ultrafine red ginseng powders

plots of both fine- and ultrafine red ginseng powders in Fig. 4(A), (B) were indicative of a type IV isotherm of Brunauer, Deming, Deming and Teller (BDDT) classification, which represents monolayer-multilayer adsorption behavior with strong affinities between adsorbate and adsorbent at the initial adsorption stage [12]. The presence of hysteresis loops are the result of capillary condensation within fine capillaries, which indicates both fine- and ultrafine red ginseng powders are mesoporous with slit-shaped pores. During vapor sorption, 90.0% RH state was

Fig. 3 Zeta-potentials of fine (○) and ultrafine (●) red ginseng powders as pH

achieved by 36.57 and 35.31% gains in weight for the fine- and ultrafine red ginseng powders, respectively. However, ultrafine red ginseng powder gained more in weight in the initial sorption period from 0 to 40% RH state, which RH range is used for the calculation of BET surface area and pore distributions (Table 1).

The specific surface area determined using the dynamic vapor sorption method is shown in Fig. 4(C). Dynamic vapor sorption is a preferred measure of specific surface area for food ingredients composed of organic materials such as fruit, vegetable, and grain because conventional nitrogen or helium adsorption methods require hyper thermal pretreatment before measurement. The specific surface areas of the fine- and ultrafine red ginseng powders were 48.72 ± 6.41 and 86.74 ± 5.96 m²/g, respectively, indicating that the ultrafine red ginseng powder containing relatively small particles has a larger specific surface area than the fine red ginseng powder containing relatively large particles. This is in good agreement with the result of the

Fig. 4 Dynamic vapor sorption (DVS) isotherms of (A) fine- and (B) ultrafine red ginseng powders and (C) corresponding specific surface areas

Table 1 Kinetics of sorption and desorption of vapor on fine- and ultrafine red ginseng powders

Target RH (%)	Fine red ginseng powder		Ultrafine red ginseng powder	
	Sorption	Desorption	Sorption	Desorption
0.0	0.00	0.00	0.00	0.00
10.0	0.85	2.15	1.97	2.93
20.0	1.23	2.95	2.63	3.81
30.0	1.53	3.67	3.12	4.45
40.0	1.93	4.63	3.63	5.57
50.0	4.43	6.51	5.47	7.34
60.0	9.27	9.54	9.72	10.09
70.0	13.82	13.89	14.13	14.32
80.0	20.79	20.88	20.81	21.81
90.0	36.57	36.57	35.31	35.31

particle size distribution profiles of the fine- and ultrafine red ginseng powders; the ball milling decreased the particle size distribution profile toward small size and produced an ultrafine powder with small-sized particles. In general, specific surface area, i.e., the total surface area of material per unit of mass, of the powder with smaller particles has larger specific surface than the powder with larger particles.

In vitro digestion study

Release profiles of ginsenoside Rg1 from fine- and ultrafine red ginseng powders in simulated gastric and intestinal fluids over time are shown in Fig. 5. Ultrafine red ginseng powder showed higher release rate of ginsenoside Rg1 (1313 ± 56 mAU s, 159 ppm) than that (1013 ± 159 mAU s, 123 ppm) of the fine one at the initial stage in the simulated gastric fluid. The increased specific surface area of the ultrafine red ginseng powder released Rg1 rapidly in the gastric fluid. As time elapsed, the release of Rg1 was still higher in ultrafine powder than in fine powder, because

Fig. 5 Release property of ginsenoside Rg1 from fine- and ultrafine red ginseng powders in simulated (A) gastric and (B) intestinal fluids

ginsenoside Rg1 was easily released from the intact cell wall structure, which was broken up rigorously by the ball milling. The release behavior of ginsenoside Rg1 was similar regardless of particle size, indicating that the release of ginsenoside Rg1 shows diffusion-type release from the particle matrix. In simulated intestinal fluid, the initial concentration of ginsenoside Rg1 in the ultrafine red ginseng powder was higher than that in the fine one, similar to the observations in simulated gastric fluid. As time elapsed, the release of ginsenoside Rg1 from both fine- and ultrafine red ginseng powders increased slightly.

In the study of Lee et al. [10], the effect of particle size of red ginseng granules on the extraction yield of ginsenosides and their inhibitory effects on LPS-induced cytokine expression was investigated. They revealed that ultrafine red ginseng granules show extraction yields two times higher than normal-sized red ginseng granules. Furthermore, Zhong et al. [6] showed a higher release rate of polyphenols and flavonoids from superfine pomegranate peel compared with the coarse one, similar to our observed trend.

From our results and previous reports, it is expected that particle size reduction and the corresponding increase in specific surface area improve the release of ginsenosides from red ginseng powder in the gastrointestinal tract and enhance its chances of absorption in the human body.

In this study, the effect of the particle size of red ginseng powders on the release property of ginsenoside Rg1 in simulated in vitro digestion condition was investigated. From particle size analysis and the SEM images, we observed the formation of smaller particle fractions in the ultrafine red ginseng powder compared with the fine one. Specific surface area determination supported this observation. The release amount of ginsenoside Rg1 did not change significantly over time. However, the ultrafine red ginseng powder released more ginsenoside Rg1 than the fine one. It is expected that the ultrafine red ginseng powder obtained by two-step pulverization could improve

the release property of ginsenosides in simulated gastrointestinal fluids compared with the fine powder owing to the formation of smaller particle fractions and corresponding increase in specific surface area. Our conclusion is that a reduction in particle size of red ginseng powder could increase its bioavailability.

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References

- Radad K, Gille G, Rausch WD. Ginseng use in medicine: perspectives on CNS disorders. *IJPT* 3: 30–40 (2004)
- Lu JM, Yao Q, Chen C. Ginseng compounds: an update on their molecular mechanisms and medical applications. *Curr Vasc Pharmacol* 7: 293–302 (2009)
- Choi KT. Botanical characteristics, pharmacological effects and medicinal components of Korean *Panax ginseng* C A Meyer. *Acta Pharmacol Sin* 29: 1109–1118 (2008)
- Lim CY, Moon JM, Kim BY, Lim SH, Lee GS, Yu HS, Cho SI. Comparative study of Korean white ginseng and Korean red ginseng on efficacies of OVA-induced asthma model in mice. *J Ginseng Res.* 39: 38–45 (2015)
- Choi KO, Lee I, Paik SYR, Kim DE, Lim JD, Kang WS, Ko S. Ultrafine *Angelica gigas* powder normalizes ovarian hormone levels and has antiosteoporosis properties in ovariectomized rats: particle size effect. *J Med Food* 15: 863–872 (2012)
- Zhong C, Zu Y, Zhao X, Li Y, Ge Y, Wu W, Zhang Y, Li Y, Guo D. Effect of superfine grinding on physicochemical and antioxidant properties of pomegranate peel. *Int J Food Sci Technol* 51: 212–221 (2016)
- Zhang M, Wang F, Liu R, Tang X, Zhang Q, Zhang Z. Effects of superfine grinding on physicochemical and antioxidant properties of *Lycium barbarum* polysaccharides. *LWT—Food Sci Technol* 58: 594–601 (2014)
- Ahn J, Lee JS, Yang KM. Ultrafine particles of *Ulmus davidiana* var. *japonica* induce apoptosis of gastric cancer cells via activation of caspase and endoplasmic reticulum stress. *Arch. Pharm. Res.* 37: 783–792 (2014)
- Zhao G, Liang X, Wang C, Liao Z, Xiong Z, Li Z. Effect of superfine pulverization on physicochemical and medicinal properties of Qili Powder. *Rev. Bras. Farmacogn* 24: 584–590 (2014)

10. Lee HC, Vinodhkumar R, Yoon J, Park SK, Lee CW, Kim HY. Enhanced inhibitory effect of ultra-fine granules of red ginseng on LPS-induced cytokine expression in the monocyte-derived macrophage THP-1 cells. *Int J Mol Sci* 9: 1379–1392 (2008)
11. Kim DH, Cho JH, Cho YJ. Anti-inflammatory Activity of Extracts from Ultra-Fine Ground *Saururus chinensis* Leaves in Lipopolysaccharide-Stimulated Raw 264.7 Cells. *J Appl Biol Chem* 59: 37–43 (2016)
12. Carmody O, Frost R, Xi Y, Kokot S. Surface characterisation of selected sorbent materials for common hydrocarbon fuels. *Surf Sci* 601: 2066–2076 (2007)