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Preparation of Nano/Submicron *Ganoderma tsugae* and Its Stability

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ABSTRACT

The preparation and stability of nano/submicrometer-sized *Ganoderma tsugae* (*G. tsugae*) was explored. The fruit body of *G. tsugae* was treated by homogenization and media-milling. Three sizes (0.8, 0.3, and 0.2 mm) of media were employed for consecutive milling. The final product was obtained by centrifugation (10,000 *g*, 10 min). Particle sizes were determined by using a laser-light particle size analyzer and further examined by using transmission electron microscopy. The removal of large particles resulted in better stability. After being stored at 25°C for 21 days, the volume-average diameter of supernatant III increased from 0.105 to 0.137 μm. Freeze-drying resulted in particle aggregation and an increase in particle size. Twenty percent of the particles in the freeze-dried supernatant III were smaller than 100 nm, with an average diameter greater than 1 μm. Nevertheless, autoclaving did not result in severe instability of supernatant III. After being stored at 25°C for 12 months, about 40% of the particles were smaller than 100 nm, with an average diameter of 373 nm. In addition, the contents of bioactive compounds such as β-(1,3)-D-glucans, crude triterpenoids, total dietary fiber and chitin in media-milled products were much greater than those present in the hot-water extract.

Key words: nano/submicron, *Ganoderma tsugae*, media milling, stability

INTRODUCTION

The fruiting body of *Ganoderma* spp. has been recognized as a valuable Chinese herb and is widely used in oriental countries due to its anti-tumor[1,2] and immuno-regulation activities[3-6]. There are more than 180 chemical components, including polysaccharides, triterpenoids, nucleic acids, ergosterols, fatty acids, proteins, peptides and trace elements[7,8], in *Ganoderma* spp. Among these, polysaccharides and triterpenoids are considered as the major bioactive compounds. Polysaccharides are major components of the fungal cell wall[9]. Branched polysaccharide, β-1,3-D-glucan, isolated by hot-water extraction, has been shown to exhibit activity against Sarcoma 180 in mice[10]. *Ganoderma* is rich in triterpenoids[11], which has been reported to exhibit physiological effects, such as anti-tumor activities and the inhibition of histamine release.

Hot-water extraction is commonly used to obtain active compounds from *Ganoderma* spp. The water-insoluble portion (more than 90% by weight) is considered as waste and discarded in most cases. Crude fiber is a major component in the discarded portion. Chitin, another component of the crude fiber in *Ganodema* spp., has been shown to exhibit physiological effects[12,13]. Re-utilization of the waste has been considered in the literature. Fiber, isolated from discarded *Ganoderma*, has been incorporated into wound dressings[14,15] and used as excipients[16].

Size reduction can improve the texture and functionality of crude fiber and also help in releasing bioactive compounds from a matrix. The release of carotenoids and soluble fiber from goji has been significantly enhanced by size reduction[17]. The rate of hydrolysis of cellulose was increased about 8 folds by reducing its size to submicrometer scale[18]. It appears that size reduction is an attractive method to increase the release of bioactive compounds and improve the utilization of the fruiting bodies of *Ganodema* spp.

Media mill is one of various types derived from stirred ball mill. It was introduced commercially in 1948 by Du Pont as "sand mill" primarily applied as a pigment grinder in the paint and lacquer industry[19]. The use of more densely

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packed and rapidly rotated media improves the efficiency of comminuting[20]. The development of a grinding chamber and high efficiency temperature-control system[21] minimizes the thermal impact on materials. Date and Patravale[22] pointed out that media milling has been one of the popular methods for preparing nanoparticles in the drug industry. Size reduction by media-milling had been successfully used to improve the solubility and bioavailability of Zn-insulin[23]. Few literatures are concerned with the application of media milling in food. Chocolate appears to be the first material to be manufactured by related technology. Alamprese *et al*.[24] reported the optimum operating conditions of a ball mill refiner for chocolate. One of the features of media milling is the preparation of hydrophobic materials at nano/submicrometer scale suspended in water with reasonable shelf life. When in a suspension, nanoparticles will not float into air and pose as a hazard to the environment and human respiratory system.

G. tsugae has been used as a supplement for its physiological effects, such as immune-regulation. Extraction is a common practice to obtain desired products. Nevertheless, there are very few literatures concerned with preparing nano/submicron particles from *G. tsugae* fruiting bodies. The objectives of this study were to explore the feasibility of preparing nano/submicron *G. tsugae* by media milling, and to investigate the stability of nano/submicrometered particles in dispersions and supernatants.

MATERIALS AND METHODS

I. *Materials*

Fresh fruiting bodies of *G. tsugae* (CCRC 36065), originating from the Culture Collection and Research Center in Taiwan, were provided by the Double Crane Enterprise Co. (Tainan, Taiwan) and stored at -40°C until use. The chemicals used in this study were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA), unless otherwise specified.

II. *Hot-Water Extract*

The fruiting bodies of *G. tsugae* were air-dried in oven at 70°C for 24 h and then pulverized using a grinder (RT-08, Rong-Tsong, Taichung, Taiwan). The ground sample (16 g, dry weight) was mixed with deionized water (1000 mL), heated at 100°C for 3 h, and then cooled to room temperature before filtration through Whatman No. 4 filter paper. The filtrate was collected as hot-water extract.

III. *Preparation of Nano/Submicron G. tsugae*

Nano/submicrometer-sized particles of *G. tsugae* were prepared according to the method of Chen *et al*.[25] A media mill (MiniPur, Netzsch-Feinmahltechnik GmbH, Selb, Germany) with a driving motor of 0.94 kW was utilized to prepare samples. Media (yttria-stabilized tetragonal zirconia, YTZ) of 0.8, 0.3 or 0.2 mm were placed in the milling chamber (200 mL) at a filling ratio of 70% (v/v). Fresh *G. tsugae* fruiting bodies were cut into small pieces (8 g dry weight) and blended with 400 mL of deionized water for 5 min. The mixture was homogenized (Polytron PT 3000, Kinematica AG, Switzerland) at 20,000 rpm for 10 min to obtain the pre-dispersion, which was further pulverized by media milling. The pre-dispersion was loaded into a jacketed-cooling tank, washed by 100 mL of deionized water, and then fed at a flow rate of 600 ± 5 mL/min into the milling chamber by a circulation pump. Therefore, the solid content was the same as that for hot-water extraction (16 g/1000 mL). The temperature of dispersion was maintained below 15°C by a circulation system. The agitation speed was set at 3,600 rpm. Milling was conducted in three-consecutive steps using 0.8, 0.3 and 0.2 mm media and the milling time was 60 min for each step. The dispersions collected from the above three steps were designated as dispersion I (0.8 mm media, 60 min-milling), dispersion II (0.3 mm media, 60 min-milling) and dispersion III (0.2 mm media, 60 min-milling), respectively. The gaps of media separator for media of 0.8, 0.3 and 0.2 mm were 0.3, 0.15 and 0.1 mm, respectively. The corresponding supernatants of the three dispersions were obtained by centrifugation at 10,000 *g* for 10 min, and designated as supernatant I, II, and III. All samples after media-milling were recognized as milled samples.

IV. *Particle Size*

The particle size distributions of dispersions and supernatants were determined by a dynamic light scattering particle size analyzer (Nanotrac 150, Microtrac Inc., Largo, USA) with a detecting range of 0.0008-6.54 μm. When large particles were present, size analysis was conducted by using a laser light diffraction particle size analyzer (LS 230, Beckman Coulter, Inc., Fullerton, CA, USA) with a detecting range of 0.04-2000 μm. Both instruments were calibrated with deionized water. Before measurements, all the samples were diluted 10 folds, subjected to mild stirring and then degassed by sonication for 5 min at 100 W and 42 kHz (Branson 3510R-DTH, Branson Ultrasonic Corp., Danbury, CT, USA). Volume-average diameters of the particles were determined by using a software provided by Microtrac Inc. and Beckman Coulter, Inc. All the measurements were conducted in triplicate.

V. *Morphology*

The presence of nano/submicron particle was confirmed using a transmission electron microscope (TEM) (JEM-1230, JEOL Co. Ltd., Japan) at a voltage of 100 kV. Nano/submicrometer-sized *G. tsugae* supernatant was diluted with deionized water. Drops of diluted aqueous suspension were spread onto a carbon-coated microscope grid (200 mesh) (01800-F, Ted Pella, Inc., Redding, CA, USA) and dried by natural air circulation at 25°C. Only the image for supernatant III was reported.

VI. *Stability Studies*

To understand the effects of processing on the stability of nanoparticles, the milled samples (including dispersion and supernatant) were further treated by different processes, including freeze-drying, freezing at -20°C for 24 h, autoclaving at 121°C for 15 min, and concentration at reduced pressure using a rotary evaporator at 40°C. The ratio of volume of original sample to volume of concentrated sample was defined as the concentration ratio and used as an index for the increase in solid content. Particle sizes of tested samples were determined and used as an index of stability during storage at 25°C.

VII. *Analysis of Bioactive Components*

(I) *β-(1,3)-D-glucans*

Selective aniline blue reaction with β-(1,3)-D-glucan was employed to determine the content of β-(1,3)-D-glucan in hot-water extracts and milled samples. The measurement was conducted according to Chang and Lu[26] with some modifications. The sample was centrifuged at 2,000 *g* for 10 min and filtered with Whatman No. 4 filter paper. The filtrate (1 mL) was added to 0.3 N NaOH (2 mL) and stirred at 25°C for 30 min. The pH of the mixture was adjusted to 11.5 ± 0.1 using 1 N HCl and the volume was made up to 10 mL by adding Na_2HPO_4-NaOH buffer containing 0.5 M NaCl (pH 11.5). The mixture (2 mL) was reacted with 0.2 mL of 0.1% (w/v) aniline blue (Ferak, Berlin, Germany) for 2 h at 25°C. The fluorescence was measured by using a fluorescence spectrophotometer (F4500, Hitachi Co., Tokyo, Japan). The excitation and emission wavelengths were set at 395 and 495 nm, respectively. Lentinan was used to establish a standard curve for calculating the contents of β-(1,3)-D-glucan in samples.

(II) *Dietary Fiber*

Total dietary fiber (TDF) was determined using nonenzymatic-gravimetric method according to AOAC (method 993.21). Freeze-dried hot-water extracts and milled samples (500 mg) were dispersed in 25 mL of deionized water at 37°C for 90 min, to which 100 mL of 95% ethanol was added to precipitate dietary fiber in an hour at 25°C. The mixture was filtered through a Pyrex fritted disc glass crucible (pore size of 40 μm), and subjected to sequential washing with 20 mL of 78% ethanol, 10 mL of 95% ethanol and 10 mL of acetone. The ethanol-insoluble residue was dried and weighed, and the weight was corrected by ash and residual protein content. The content of TDF was calculated as follows:

$$TDF = (Wr - P - A) / Ws \quad (1)$$

where Wr = weight of residue (mg), P = weight of protein in residue (mg), A = weight of ash in residue (mg), and Ws = weight of sample (mg).

(III) *Chitins*

The content of chitin in the sample was determined as glucosamine by colorimetric method[27]. Samples (400 mg) were hydrolyzed with 200 mL of 6 N HCl at 100°C for 4 h, which resulted in the depolymerization of polysaccharides and deacetylation of acetylglucosamine units, and free glucosamine. After cooling to 25°C, the hydrolysates were filtered through Whatman No. 4 filter paper. The filtrate (1 mL) was evaporated to dryness at 45-50°C at reduced pressure. The dried hydrolysate was then dissolved in deionized water to prepare a diluted hydrolysate solution containing 5-15 μg of glucosamine hydrochloride per mL of solution. The diluted hydrolysate solution (1 mL) was added with 0.25 mL of 4% acetylacetone (4% acetylacetone in 1.25 N Na_2CO_3, v/v) and heated at 90°C for 1 h. After cooling, 2 mL of ethanol was added to dissolve the precipitates with shaking. Then, 0.25 mL of Ehrlich reagent (1.6 g of *N*-*N*-dimethyl-*p*-aminobenzaldehyde in a 30 : 30 mL mixture of ethanol and concentrated HCl) was added to form color. The absorption at 530 nm was measured. A standard curve was established by using glucosamine hydrochloride (5-50 μg/mL) for determining the contents of chitin, calculated as 1,4-anhydro-*N*-acetyl-2-deoxy- D-glucopyranose equivalent, in the samples.

(IV) *Crude Triterpenoids*

The content of crude triterpenoids was determined according to the method reported by Ma *et al*.[28] with some modifications. Hot-water extracts and milled samples (100 mL) were freeze-dried and then added into 100 mL of 95% ethanol with stirring at 25°C for 48 h. The mixture was then filtered through Whatman No. 1 filter paper and the filtrate was dried at 50°C in a vacuum oven. The dried powder was suspended in 10 mL of a solution of deionized water and $CHCl_3$ (1 : 1) with vigorous agitation for extraction. Then, 5 mL of chloroform layer was obtained from the mixture and added with 5 mL of saturated $NaHCO_3$ aqueous solution for partition extraction. The alkaline layer (5 mL) was obtained and added with 5 mL of $CHCl_3$ for partition extraction. Again, 5 mL of chloroform layer was obtained and dried at 50°C using a vacuum oven. The dried powder was weighed and considered non-acidic triterpenoids. The alkaline layer obtained above was added with 6 N HCl to adjust the pH to 2-3.5 mL of $CHCl_3$ was added for partition extraction. The chloroform layer (5 mL) was obtained and dried at 50°C using a vacuum oven. The dried powder was weighed and considered as acidic triterpenoids. The content of crude triterpenoids was the sum of non-acidic triterpenoids and acidic triterpenoids.

(V) *Statistical Analysis*

Data were reported as means of measurements conducted in triplicate. One-way analysis of variance (ANOVA) was used to determine the significance of treatment using SPSS software (version 12.0, SPSS Inc., Chicago, IL, USA),

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followed by Duncan's multiple comparison test. Differences were considered as statistically significant when the *P* value was less than 0.05.

RESULTS AND DISCUSSION

I. *Particle Size of Dispersions*

The particle size of pre-dispersion ranged from 1.4 to 1314 μm with a volume-average diameter of 415 μm (Figure 1A). Most of the particles ranged from 300 to 1000 μm, which resulted in rapid precipitation. After further grinding by media-milling, the largest particle in dispersion I (ranging from 0.04 to 105.9 μm) was reduced to 106 μm and particles smaller than 1 μm were observed (Figure 1B). The volume-average diameter was 11.1 μm. More nano/submicrometer-sized particles were found in dispersion II (Figure 1C) after the second milling using 0.3-mm media. The particles that were greater than 1 μm had been greatly reduced and the largest particle was found to be 55 μm with a volume-average diameter of 6.5 μm and a size range of 0.04-55.1 μm. After further milling by using 0.2-mm media, more particles smaller than 1 μm were present in dispersion III (Figure 1D). The largest particle was further reduced to about 3 μm with a size ranging from 0.04 to 3.1 μm. The volume-average diameter (0.79 μm) had reached nano/submicrometer scale. The data demonstrated that it was feasible to prepare nano/submicron *G. tsugae* by media milling. It appeared that 1 μm was a major cut-off for size distribution, which was similar to the literature findings[18,21]. For example, there existed two groups in dispersion III. One was in the range of 0.04-1 μm and the other one was in the rang of 1-3.1 μm. The volume percentages of particles in nano/submicrometer scale (≤ 1 μm) were 70% (v/v), among which particles in nanoscale (≤ 100 nm) were 6% (v/v).

II. *Particle Size and Morphology of Supernatants*

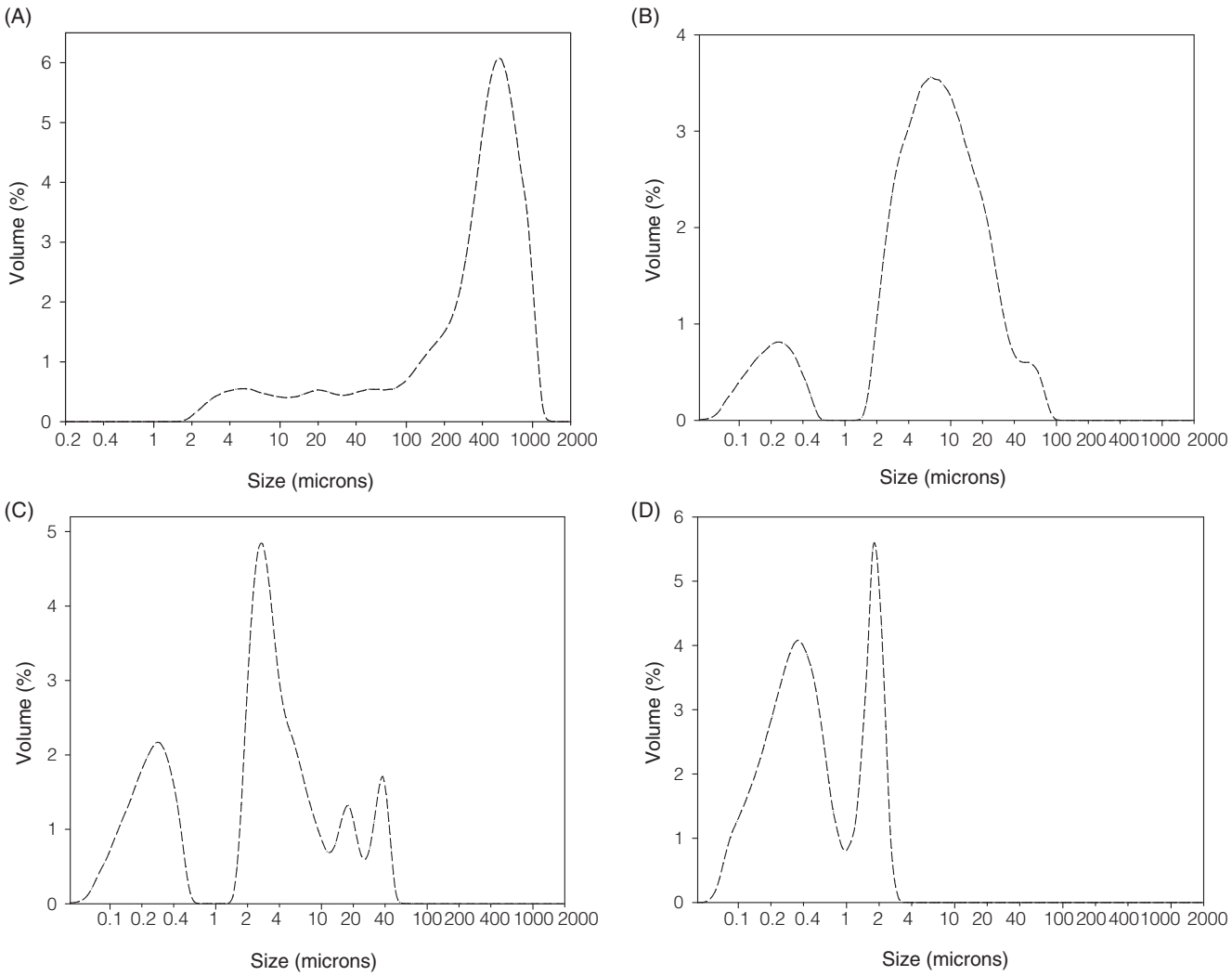

Figure 1. Particles size distributions of *G. tsugae* fruiting bodies dispersions. (A) coarse pre-dispersion by homogenization; (B) dispersion I; (C) dispersion II; and (D) dispersion III.

The supernatant appeared to be transparent and tawny without precipitates. It indicated the presence of ultrafine particles in the supernatant. For example, the sizes of particles in supernatant III ranged from 28 to 578 nm with a volume-average diameter of 105 nm (Figure 2A). The percentage of nanoparticles ($d_p \leq 100$ nm) was increased to 67% (v/v). The sizes of particles in supernatant III were confirmed by TEM (Figure 2B). Most of the particles were spherical in shape with sizes in the range of 20-100 nm. Although the size of each single particle was smaller than 100 nm, the particles were attached to each other and formed string-like structures, probably due to the high surface area and *van der* Waals forces. Particle size analysis and TEM confirmed that it was feasible to prepare nano/submicron *G. tsugae* particles using media milling.

III. *Stability of Supernatant*

It appeared that supernatants were stable as indicated by a slight change in size during storage at 25°C. The volume-average diameter of supernatant I increased from 0.158 to 0.341 μm (Table 1) after being stored for 21 days. There was no significant increase in the volume-average diameters of supernatant II and III during 21 days of storage. As supernatant III yielded the smallest volume-average diameter (0.105 μm) and was stable during storage, it was employed as an example for the stability study.

Freeze-drying resulted in the aggregation of particles. After being dispersed in water, the freeze-dried powder from supernatant III yielded about 20% of particles smaller than 100 nm with a volume-average diameter greater than 1 μm (Figure 3). The largest particle was found to be about 6 μm, which was much greater than 0.578 μm in Figure 2A. Nevertheless, autoclaving did not result in a significant increase in the particle size of supernatant III. All the particles were smaller than 1 μm after autoclaving (Figure 4A). There were about 50% (v/v) of particles that were smaller than 100 nm, indicating a 10% (v/v) decrease due to autoclaving compared with that before autoclaving. The size of the largest particle increased from 0.6 to 0.8 μm, indicating that supernatant III (volume-average diameter of 0.140 μm) was comparatively stable for autoclaving. After being stored at 25°C for 4 weeks, few particles were greater than 1 μm with a slight increase in volume-average diameter (0.165 μm) (Figure 4B). There still existed about 50% (v/v) of particles that were smaller than 100 nm. Twelve months of storage resulted in a greater number of particles with a size greater than 1 μm, but there were about 40% (v/v) of particles smaller than 100 nm with a volume-average diameter of 373 nm (Figure 4C). The largest particle was about 3 μm. The data indicated that autoclaved

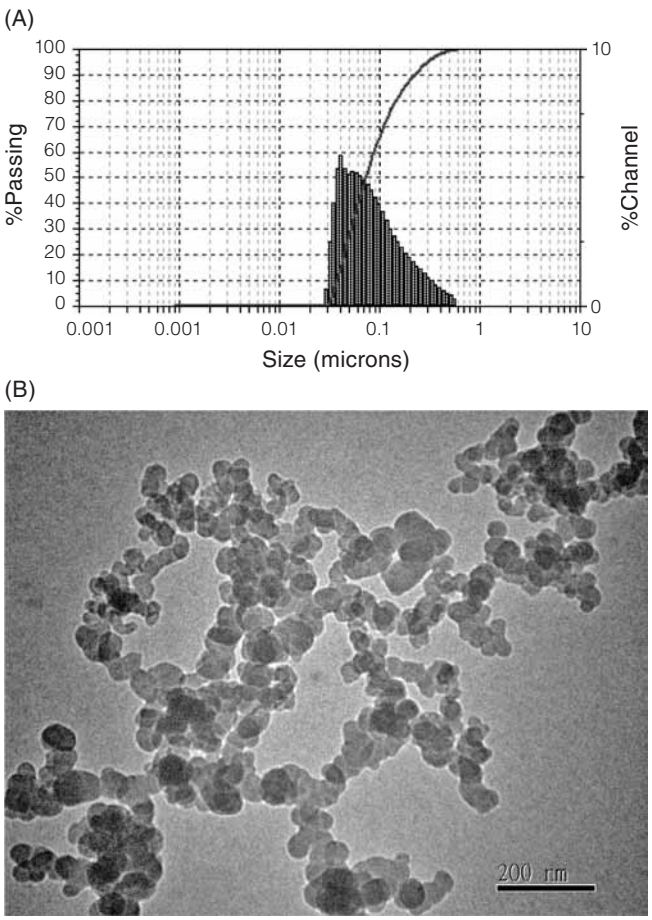

Figure 2. (A) Particle size distribution of supernatant III; (B) Transmission electron micrograph of particles in supernatant III.

Table 1. Volume-average diameters[g,h] of supernatants stored at 25°C for 0, 5 and 21 days

Supernatant	Volume-average diameter, μm		
	0 day	5 days	21 days
I	0.158 ± 0.022[a]	0.236 ± 0.010[b]	0.341 ± 0.017[c]
II	0.149 ± 0.023[a]	0.149 ± 0.013[a]	0.153 ± 0.010[a]
III	0.105 ± 0.007[a]	0.133 ± 0.015[b]	0.137 ± 0.012[b]

[g] Mean ± standard deviation (n = 3).
[h] Different lower case letters (a, b and c) in the same row indicate significant difference at $p<0.05$ analyzed by Duncan's multiple range test.

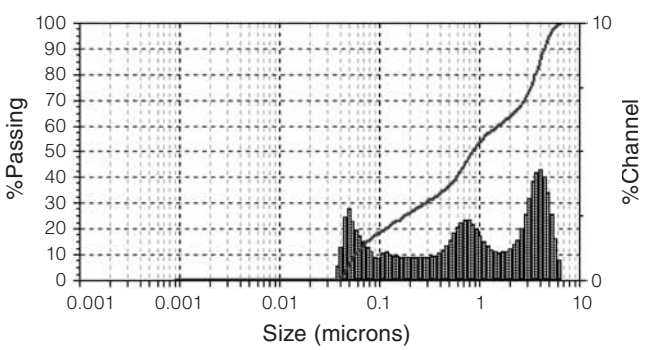

Figure 3. Particle size distribution of rehydrated freeze-dried powder from supernatant III. The particle size ranged from 0.04 to 6.54 μm with an average diameter of 1.71 μm.

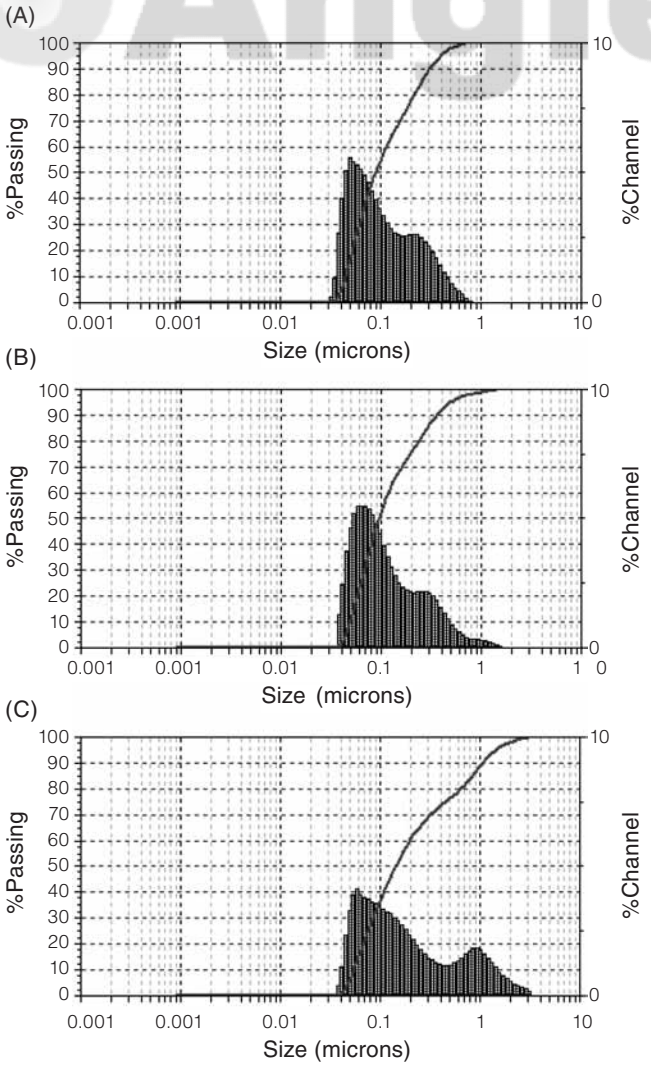

Figure 4. Particle size distributions of supernatant III after autoclaving and storing at 25°C for (A) 0 day, (B) 4 weeks, and (C) 12 months.

supernatant could be stored for a year with reasonable stability.

Concentration was also employed to evaluate the stability of supernatant III. When the concentration ratio was 4 with a solid content of 13.6 mg/mL, all particles were still smaller than 1 μm (Table 2). The corresponding volume-average diameter was 0.140 μm, which was greater than the original (0.105 μm). At a concentration ratio smaller than 4, the supernatant was so dilute (13.6 mg/mL) that particle aggregation was minimized, resulting in good stability. As higher concentration ratios were approached, particles aggregated to form larger clusters, which were measured as particles greater than 1 μm. A concentration ratio of 8 resulted in 5% of particles greater than 1 μm with a volume-average diameter of 0.351 μm. Further concentration resulted in severe aggregation of particles with a lot of particles greater than 6 μm, limiting the determination of particle size distribution by Nanotrac 150. Thus, the volume-average diameter and percentage of particles in nano/submicron scale were not

Table 2. Effects of concentration ratio on the particle size of supernatant III

Concentration ratio[g]	Volume-average diameter (μm)[h,i]	Distribution range (μm)	Volume percentage of particles smaller than 1 μm (%)
0 (3.4)	0.105 ± 0.007[a]	0.030 - 0.578	100
2 (6.8)	0.141 ± 0.011[b]	0.036 - 0.750	100
4 (13.6)	0.140 ± 0.008[b]	0.030 - 0.972	100
8 (27.2)	0.351 ± 0.022[c]	0.033 - 2.120	95
12 (40.8)	nd[j]	0.111 - > 6.54	nd
20 (68.0)	nd	0.859 - > 6.54	nd

[g] Concentration ratio = volume of original sample/volume of concentrated sample. The number in parenthesis is the corresponding concentration (mg/mL).
[h] Mean ± standard deviation (n = 3).
[i] Different lower case letters (a, b and c) in the same column indicate significant difference at $p < 0.05$ analyzed by Duncan's multiple range test.
[j] nd: not determined.

Table 3. Solid contents in samples prepared from *G. tsugae* fruiting bodies

Sample	Solid content (mg/mL)[g]
Hot water extract	1.7 ± 0.2
Pre-dispersion	20.1 ± 0.2
Dispersion I	16.3 ± 0.6
Dispersion II	13.7 ± 0.4
Dispersion III	10.5 ± 0.3
Supernatant I	1.8 ± 0.4
Supernatant II	2.8 ± 0.2
Supernatant III	3.4 ± 0.1

[g] Mean ± standard deviation (n = 3)

determined for the concentration ratios of 12 and 20. The data indicated that when the solid content was greater than 27.2 mg/mL, the percentage of particles greater than 1 μm increased with solid content.

IV. *Solid Content and Bioactive Compounds*

Obviously, the solid content (20.1 mg/mL) in pre-dispersion would be the greatest among all samples. As media-milling was conducted in series, it was reasonable to observe a decrease in the solid contents of dispersions I, II, and III (Table 3). The yield of each milling step was from 77 to 85%, as some solids encaged in the milling chamber. Nevertheless, supernatant III had the highest solid content (3.4 mg/mL) among the three supernatants. As discussed above, supernatant III exhibited the smallest particle size among the three supernatants, indicating that size reduction enhanced the solid content in the final product. All three supernatants had solid contents higher than that of the hot-water extract (1.7 mg/mL). The increase in solid content might include water-soluble and water-insoluble components

Table 4. Contents[g] of chitin, TDF and crude triterpenoid in hot-water extract and supernatant III prepared from *G. tsugae* fruiting bodies

Sample	Chitin (mg/g)	TDF[h] (mg/g)	Crude triterpenoid (μg/mL)
Hot water extract	15.1 ± 0.0	94.5 ± 4.2	244.0 ± 15.7
Supernatant III	26.7 ± 0.4	145.2 ± 3.4	321.1 ± 7.1

[g] Mean ± standard deviation (n = 3).
[h] Total dietary fiber (AOAC 993.21).

Table 5. Contents of β-1,3-D-glucan in hot-water extract, supernatants of media-milled *G. tsugae* fruiting bodies and commercial *Ganoderma* drink products

Sample	Concentration of β-1,3-D-glucan (μg/mL)[g]
Hot water extract	10.3 ± 0.0
Supernatant I	9.6 ± 0.4
Supernatant II	39.8 ± 0.3
Supernatant III	61.9 ± 0.4
Product 1[h]	2.3 ± 0.1
Product 2	10.0 ± 0.1
Product 3	20.2 ± 0.1
Product 4	7.9 ± 0.1
Product 5	2.5 ± 0.0

[g] Mean ± standard deviation (n = 3).
[h] Commercial *Ganoderma* drink products.

(nano/submicron particles). Thus, the contents of chitin, total dietary fiber (TDF) and crude triterpenoids in supernatant III were higher than those in the hot-water extract (Table 4). The major bioactive components in *G. tsugae* fruiting body are polysaccharides and triterpenoids[9,11]. The content of β-1,3-D-glucan has been used as an index for the presence of polysaccharides. The contents of β-1,3-D-glucan in most of the commercial products in Taiwan were lower than 10 μg/mL (Table 5). In this study, only one commercial product contained 20.2 μg/mL of β-1,3-D-glucan. The content of β-1,3-D-glucan in the hot-water extract (10.3 μg/mL) was close to those of commercial products. As discussed above, supernatants II and III had higher solid contents than supernatant I, and thus had more β-1,3-D-glucan. The content of β-1,3-D-glucan in supernatant III was 6 folds that of the hot-water extract. The data illustrated that media-milling raised the contents of chitin, crude triterpinoid and β-1,3-D-glucan in the final products. In addition, the size reduction into nano/submicrometer scale resulted in smoother texture, which is worthy of further studies.

CONCLUSIONS

The data demonstrated that it was feasible to prepare a dispersion of nano/submicron *G. tsugae* fruiting bodies by media milling. Nevertheless, the presence of both small and large particles resulted in the instability of dispersions. After centrifugation, the supernatant was stable after autoclaving and storing at 25°C for 12 months. High solid content affected the stability of milled products. When the solid content was higher than 27 mg/mL, aggregation occurred due to an increase in particles greater than 1 μm. The contents of β-1,3-D-glucan in supernatants were greater than that in the hot-water extract. The results demonstrated that media milling could enhance the release of bioactive compounds and result in a smoother texture.

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