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Preparation of Nano/Submicrometer Yam and Its Benefits on Collagen Secretion from Skin Fibroblast Cells

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ABSTRACT: Nano/submicrometer-scaled yam particles have been prepared by using media-milling. The particle size of media-milled yam was confirmed by the laser light scattering method and scanning electron microscopy. Influences of media-milled yam on skin fibroblast cells (WS1) were evaluated. The size reduction did not significantly alter the proximate composition, and the presence of nanoparticles was not toxic to WS1 cells. The contents of bioactive compounds (diosgenin, stigmasterol, and β-sitosterol) were significantly increased by media-milling, which enhanced the secretion of hTGF-β and inhibited the formation of MMP-1. Thus, the collagen secretion from WS1 was significantly increased by size reduction. Diosgenin was employed as a positive control. Nevertheless, media-milled yam exhibited greater effects on WS1 cells than diosgenin. It appeared that both diosgenin and size reduction were helpful for enhancing the secretion of collagen by WS1 cells. In addition, the irritancy of yam was eliminated by media-milling.

KEYWORDS: *collagen secretion, yam, skin fibroblasts, media-milling*

INTRODUCTION

Yam is a common name of *Dioscorea* spp., which is a perennial trailing rhizome plant, and is generally utilized as a staple food or in Chinese medicine in Asia.[1] Several phytochemicals, including phytosterol, steroidal saponins, mucopolysaccharides, glycoprotein, and minerals, in yam have been considered beneficial to health.[2,3] Among those, saponins have caught researchers' attention due to their effects on menopause syndrome without toxicological issues.[4] Diosgenin, an aglycon of steroidal saponins, has been shown to reduce cholesterol levels,[5] to control hyperlipidemia,[6] and to reduce amyloid plaques and thus to improve Alzheimer's disease.[7] Diosgenin has also been found to be a weak phytoestrogen, and it inhibits proliferation of human breast carcinoma cells (MCF-7 cells) via G0/G1 arrest and leads to apoptosis by activating p53 and down-regulating Akt and BRCA-1 expressions.[8,9] Yam has been considered a major plant resource of diosgenin, which is a natural steroidal saponin used for hormonal products.[10] A method which raises diosgenin content from bioresources would be an attractive approach for utilizing whole plants.

Studies of postmenopausal women indicate that the loss of estrogen is associated with symptoms including dryness, atrophy, fine wrinkles, epidermal thinning, and declining dermal collagen content.[11] The identification of estrogen receptor (ER) in skin fibroblasts and keratinocytes provides the evidence that estrogen plays an essential role in preventing the skin deterioration[12] of females, particularly for postmenopausal women.[13,14] Estrogen (17β-estradiol) has been shown (in vitro study) to induce proliferation of human dermal fibroblasts and further increases the secretion of collagen, glycosamminiglycans, and proteoglycans.[15] The effect has been attributed to the regulation of transforming growth factor (hTGF-β) and other relative growth factors.[16] Diosgenin has been found to increase the secretion of collagen from ovariectomized estrogen-deficient rats[17] and the dermal thickness of hairless mice due to its structure similar to that of 17β-estradiol.[18]

Reducing the size to the nano/submicrometer scale is believed to enhance the bioavailability of nutrients. Liao et al.[19] have reported that nano/submicronization enhances the transport and absorption of lignan glycosides by Caco-2 cells. Size reduction also enhances the bioactivities of goji berry on gene expressions.[20] One economical way to reduce the size is to employ a media mill, one of various types derived from stirred-ball mills.[21] Date and Patravale[22] have pointed out that media-milling is a popular method to prepare nanoparticles in the drug industry. Few papers in the literature are concerned with the application of media-milling in food. The purpose of media-milling is to prepare hydrophobic materials at a nano/submicrometer scale, suspended in water and having a reasonable shelf life. Understanding the effect of size reduction on the secretion of collagen from a fibroblast cell would be helpful for applying nanotechnology in functional food or nutricosmetics by utilizing edible resources. The objective of this study was to explore the feasibility of preparing nano/submicronization of yam and its potential benefits on collagen secreted from skin fibroblast cells.

MATERIALS AND METHODS

Samples. *Dioscorea japonica* Thunb var. *pseudojaponica* Yamamoto, a native yam in Taiwan, was purchased from Reefung Farmers' Association (Keelung City, Taiwan). Yams (with peel) were minced by using a Waring blender (7012S, Waring, Torrington, CT) and freeze-dried by using the FreeZone 18 L freeze-dry system (Labconco Co., Kansas City, MO). Then a Pulverisette 14 rotor speed mill (Fritsch GmbH, Idar-Oberstein, Germany) was employed to grind the dried

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product, by impact and shearing, to pass a 20-mesh (ASTM) sieve. The ground dried yam powder was designated as the starting material and stored in a desiccator at 25 °C until use.

Media-Milled Yam Preparation. Nano/submicrometer yam particles were prepared using a media mill. Starting material (10 g) was mixed with distilled water (the total mass was 400 g) and further ground using a high-speed blender (PT 3100, Kinematica, Lucerne, Switzerland) at 10 000 rpm for 10 min to obtain blended yam, which was further processed by a two-step media-milling to obtain a nano/submicrometer yam suspension. Media (yttria-stabilized tetragonal zirconia, YTZ) of 0.8 or 0.3 mm were placed at a 70% (v/v) filling ratio in a milling chamber (200 mL) of a media mill (Minipur, Netzsch-Feinmahltechnik GmbH, Germany) with a driving motor of 0.94 kW. The blended yam (400 g) was loaded into a jacket-cooling tank and then fed at a flow rate of 300 mL/min into the milling chamber by a circulation pump. During milling, particles smaller than the gap of the media separator were brought out into the stirred tank for cooling by a circulation system. The temperature of the suspension was thus maintained below 25 °C during milling. The agitation speed was set at 3000 rpm. The milling was conducted with 0.8 mm media for 30 min, and then 0.3 mm media was employed for 60 min. The total milling time was 90 min.

Particle Size Distribution. The particle size distribution of the sample was determined by using a laser light diffraction particle size analyzer (LS 230, Beckman Coulter, Fullerton, CA) with a detection range of 0.04−2000 μm. The instrument was calibrated with deionized water. All of the samples were diluted, subjected to mild stirring, and then degassed by sonication (Branson 3510R-DTH, Branson Ultrasonic Corp., Danbury, CT) for 5 min at 100 W and 42 kHz. Average diameters (in volume and number) of the particles were obtained using software with LS 230 (Beckman Coulter version 3.29). All the measurements were done in triplicate, and the average data were reported.

Morphology. An optical microscope (Optiphot-Pol, Nikon, Japan) was employed to observe the morphology of the starting material and blended yam. The microstructure of blended and media-milled yam was examined by scanning electron microscopy (SEM) (Hitachi S-4800, Hitachi Co. Ltd., Tokyo, Japan). Critical-point drying was employed to prepare samples for SEM observation. Yam suspension (blended or media-milled, 2 mL) was mixed with 18 mL of 75% ethanol and then was evaporated to 2 mL at vacuum conditions. The replacement process was repeated twice using 95% ethanol. Final replacement was conducted using 99% ethanol, and solids were suspended in 2 mL of 99% ethanol, which was further dried using liquid CO_2 in a critical-point drying apparatus (Hitachi HCP-2, Hitachi Co. Ltd., Tokyo, Japan) at 31.1 °C and 73.9 bar. After being dried, the sample was attached on an SEM stub using double-backed cellophane tape. The stub and sample were coated with gold−palladium and were examined and photographed at 15 kV.

Composition Analysis. The contents of crude protein, crude fat, crude ash, and moisture were measured using the methods of the Association of Official Analytical Chemists (AOAC).[23]

Analysis of Steroidal Saponins. The contents of diosgenin, β-sitosterol, and stigmasterol in the samples were determined according to the report of Yang et al.[24] with slight modification. Sample (2 g solid) was mixed with methanol to a 90% concentration of methanol and then stirred at 25 °C for 24 h. The mixture was filtered through Watman no. 1 filter paper. Solvent in the filtrate was removed using a rotary evaporator at 40 °C. The residue was then resuspended in 20 mL of deionized water and partitioned with 20 mL of 1-butanol three times to yield saponin extract with a total volume of 60 mL. 1-Butanol was removed by a rotary evaporator at 40 °C. The dried extract was then dissolved in 3 mL of methanol and filtered through a 0.45 μm filter for analysis by using HPLC (PU-2080, Jasco, Tokyo, Japan) with a Chromolith Performance RP-18e column (100 mm × 4.6 mm) at 35 °C and a UV/vis detector (UV-2075, Jasco). The injection volume was 20 μL. A mobile phase of 90% methanol was eluted at 1.0 mL/min for 30 min. The absorbance at 205 nm was measured and recorded. Data were processed using the JASCO Chrompass Chromatography Data System program.

Cell Viability of WS1. The alamarBlue assay (AB or resazurin assay) was employed to evaluate the effects of blended and media-milled yam on the viability of WS1 cells (human skin fibroblastoid cell line, ATCC CRL-1502) according to the method of Benavides et al.[25] WS1 cells were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidified 95% air/5% CO_2 environment. Up to 80% confluence, cells were treated with trypsin and harvested by centrifugation (1000 rpm, 5 min). Cells were counted using trypan blue dye exclusion assay, and an equal number of cells (5×10^4 cells/mL) were transferred to a 96-well plate (Greiner Bio-One GmbH, Frickenhausen, Germany). After 24 h for allowing cell attachment, the medium was removed, and WS1 cells were cocultured with various doses of yam sample, which was filtered through a 0.45 μm membrane before being added to the medium. After overnight incubation, the medium was removed, and AB solution (100 μL) was added to the cell culture with a final concentration of 20 ppm resazurin. The plate was then incubated for 3 h for the optimal transition of resazurin to resofurin. For the blank test, resazurin was added to the plate without cells. A control group (designated as SF) was conducted by following the procedures above without FBS and yam samples. The absorbance of the wells at 540 and 630 nm was determined using a spectrophotometer (Emax, Molecular Devices, Sunnyvale, CA). The experiments were conducted in triplicate. The number of viable cells was obtained from the dye reduction and was expressed as a percentage of AB reduction.[26] WS1 cell viability was equivalent to the AB reduction (%), which was calculated as follows:

$$\text{AB reduction (\%)} = \frac{\varepsilon_{OX}\lambda_2(A\lambda_1) - \varepsilon_{OX}\lambda_1(A\lambda_2)}{\varepsilon_{RED}\lambda_1(A'\lambda_2) - \varepsilon_{RED}\lambda_2(A'\lambda 1)}$$

where ε_{OX} = molar extinction coefficient of the alamarBlue oxidized form, ε_{RED} = molar extinction coefficient of the alamarBlue reduced form, A = absorbance of test wells, A' = absorbance of the blank test, λ_1 = 540 nm, and λ_2 = 630 nm.

Secretion of Collagen. WS1 cells were seeded in 12-well plates at 5×10^4 cells per well and incubated in DMEM with 10% FBS in a 5% CO_2 atmosphere at 37 °C for 24 h. After 24 h for allowing cell attachment, the medium was removed, and WS1 cells were cocultured with various doses of yam sample, which was filtered through a 0.45 μm membrane before being added to the medium. Culture medium was collected for determining the collagen content by the staining method.[27] Sirius red dye (1 mg/mL in saturated aqueous picric acid) was mixed with culture medium (10:1, v/v). After 30 min, the Sirius red dye−culture medium mixture was centrifuged at 10000g for 10 min. The fluid was quickly removed, and the pellet was dissolved in 0.2 mL of 0.1 N NaOH at 25 °C for 30 min. The dyed solution was transferred to 96-well plates, and the optical density (OD) was measured at 540 nm against 0.1 N NaOH as a blank. Collagen I was employed as a standard for the measurements.

Growth Factors and Metalloproteinase. Similar to the procedures described above for preparing a cell culture for determining the collagen content, culture medium was collected for further analysis. The contents of human transforming growth factor β (hTGF-β), human epidermal growth factor (hEGF), and metalloproteinase-1 (MMP-1) were determined by using commercial ELISA kits (R&D Systems, Minneapolis, MN) according to literature reports.[28−30]

Statistical Analysis. Data was reported as the mean of experiments conducted in triplicate. One-way analysis of variance (ANOVA) was used to determine the significance of treatment using the Statistical Analysis System (SAS version 9.1, SAS Institute Inc., Cary, NC), followed by a Duncan's multiple-comparison test. Differences were considered as statistically significant when the P value was <0.05.

RESULTS AND DISCUSSION

Morphology of Yam. It has been recognized that yam peel causes irritant contact dermatitis with a symptom of red and swollen skin. Raphides, needle-like $CaC_2O_4 \cdot H_2O$ crystals

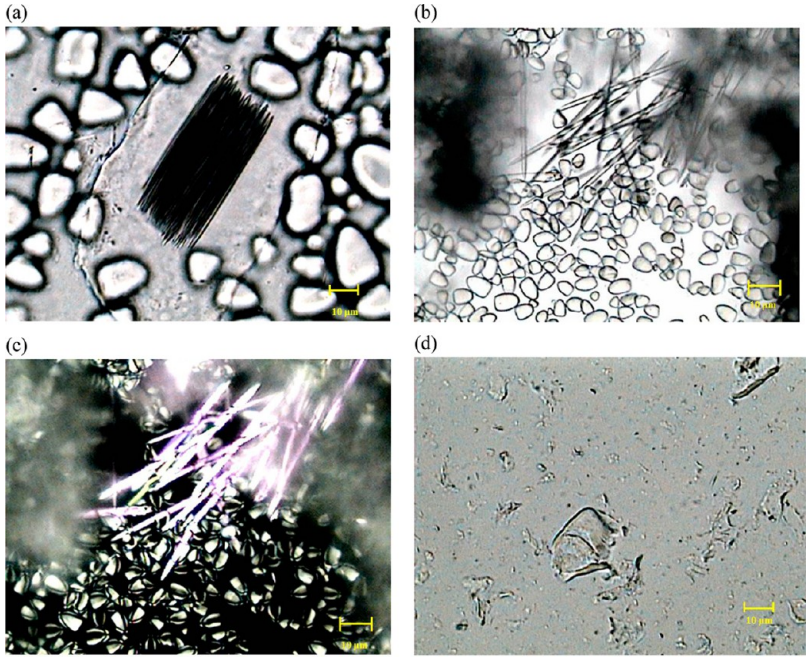

Figure 1. Optical microscopy of a raphide crystal in (a) tissue at the interface between the tuber and peel, (b) starting material (before freeze-drying), (c) starting material (before freeze-drying) under polarized light, and (d) media-milled yam (no raphides found).

sharpened at both ends, have been identified as the irritant factor from plants such as *Agave tequilana*[31] and kiwifruit.[32] In a tissue taken from the interface between the peel and tuber, there existed bundles of raphides (with a length of 60−70 μm) within a crystal idioblast cell (Figure 1a), which was similar to the report of Kawasaki et al.[33] There existed raphides in both rehydrated starting material and blended yam, which were irritants to skin. For example, individual raphide was observed in starting material (before freeze-drying) (Figure 1b). It was not surprising to see breakup of raphide bundles due to a shearing force. However, the shearing force exerted by the high-speed blender did not break the structure of the raphide itself. A chunk of black material was considered as the peel. The birefringence of raphide and starch granules (Figure 1c) was revealed by using polarized light. The raphide appeared to be a crystal. There were no raphides found in media-milled yam (Figure 1d), which was not an irritant to skin. Thus, the irritancy of yam was eliminated by media-milling. In addition, a broken starch granule was observed.

Particle Size Distribution and Microscopic Observation. The blended yam yielded a volume-average diameter of 186 ± 197 μm (Figure 2a) with a number-average diameter of 6.59 ± 6.58 μm (Figure 2b). The large deviations in average diameters were due to a wide size distribution from 2 to 825 μm. The presence of large particles resulted in rapid precipitation. About 48% (volume-based) of the particles were smaller than 100 μm. The volume-average diameter of the media-milled yam suspension was found to be 19.6 ± 13.5 μm (Figure 2c) with a number-average diameter of 3.29 ± 2.25 μm (Figure 2d). All particles were smaller than 100 μm. In number size distribution, only a few particles were greater than 10 μm. The volume-average diameter of the supernatant was determined to be 0.77 ± 0.863 μm (Figure 2e) with a number-average diameter of 0.1 ± 0.059 μm (Figure 2f). The data indicated that there were only a few particles greater than 1 μm as presented in Figure 2e. The results illustrated that nano/submicrometer yam particles were obtained by using media-milling.

As described previously, raphides existed in the blended yam observed by optical microscopy. Images without raphides and peels were selected for blended yam when SEM was employed. There existed large particles (Figure 3a), which appeared to be starch granules. In addition, there were some clusters, which could be mucilage.[34] Through a sequential enlargement of the cluster (Figure 3b), some particles were smaller than 100 nm (Figure 3c). There were stringlike materials linking particles together and forming clusters. Therefore, no particles smaller than 1 μm were detected by laser light diffraction. After media-milling, there were no particles greater than 1 μm observed (Figure 3d). In other words, starch granules were broken into small particles.[35] Part of the particles were dispersed well (Figure 3e), and part of the particles aggregated (Figure 3f). Those particles were smaller than 100 nm, which were similar to the natural nanoparticles revealed in Figure 3c, but with no linking materials. This indicated that media-milling did not break those natural nanoparticles.

Contents of Bioactive Compounds. The blended and media-milled yam suspensions appeared to have similar compositions, except crude protein and soluble fiber (Table 1). It was not surprising to observe this since the starting materials were the same. The difference in crude protein between blended and media-milled yam was about 5.3%. Neverleless, the content of soluble fiber in media-milled yam was about 2 times that in blended yam. More impacting and shearing forces resulted in degradation of fiber and thus generated more soluble fibers. Media-milling also resulted in more bioactive compounds, including diosgenin, stigmasterol, and β-sitosterol (Table 2), due to complete breakage of the material matrix. The contents of diosgenin and β-sitosterol in blended yam were slightly greater than those in the starting material. After centrifugation (10000g, 15 min), most of the active compounds were present in the precipitate. About 12% solids of blended yam were present in the supernatant with 18.5

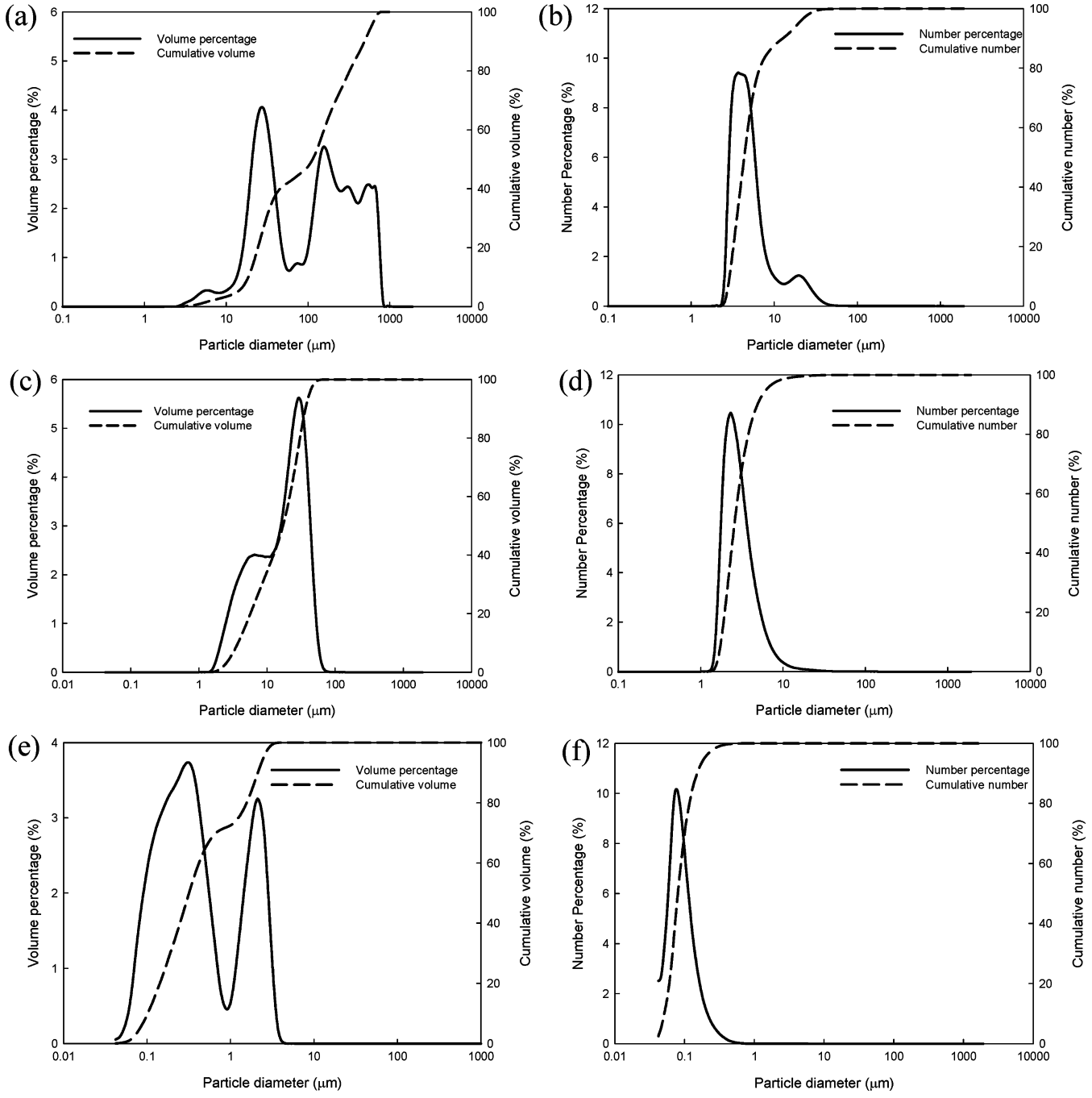

Figure 2. Particle size distribution of blended yam in (a) volume and (b) number, media-milled yam in (c) volume and (d) number, and the supernatant of centrifuged media-milled yam in (e) volume and (f) number.

μg/g diosgenin. There was no stigmasterol or β-sitosterol detected in the supernatant. After media-milling, 45% solids in media-milled yam were present in the supernatant with three bioactive compounds. Compared with the supernatant from the blended yam, the content of diosgenin in the supernatant of the media-milled yam was more than triple. The contents of stigmasterol and β-sitosterol became measurable in the supernatant of media-milled samples. The data illustrated that media-milling could be utilized as an alternative method for increasing the contents of bioactive compounds and dietary fibers. For example, stigmasterol was undetectable in blended samples. Nevertheless, there was more than 22 μg/g stigmasterol in media-milled samples. Compared with the blended sample, there existed a more than 50% increase in the content of β-sitosterol in the supernatant of the media-milled sample. The content of diosgenin has been found to range from 3 to 26 μg/g dry weight.[36] The species, age, and planting conditions result in a change of the diosgenin content. [37] It appeared that media-milling resulted in a remarkable increase in the diosgenin content.

Cell Viability. Both blended and media-milled yam did not affect the viability of skin fibroblast cells (WS1) at the addition level in this study (Figure 4). Being employed as positive controls, both L-ascorbate and diosgenin (at 5 and 10 μg/mL) did not affect the viability of WS1. Although there existed a minor decrease in viability when blended yam was added at low

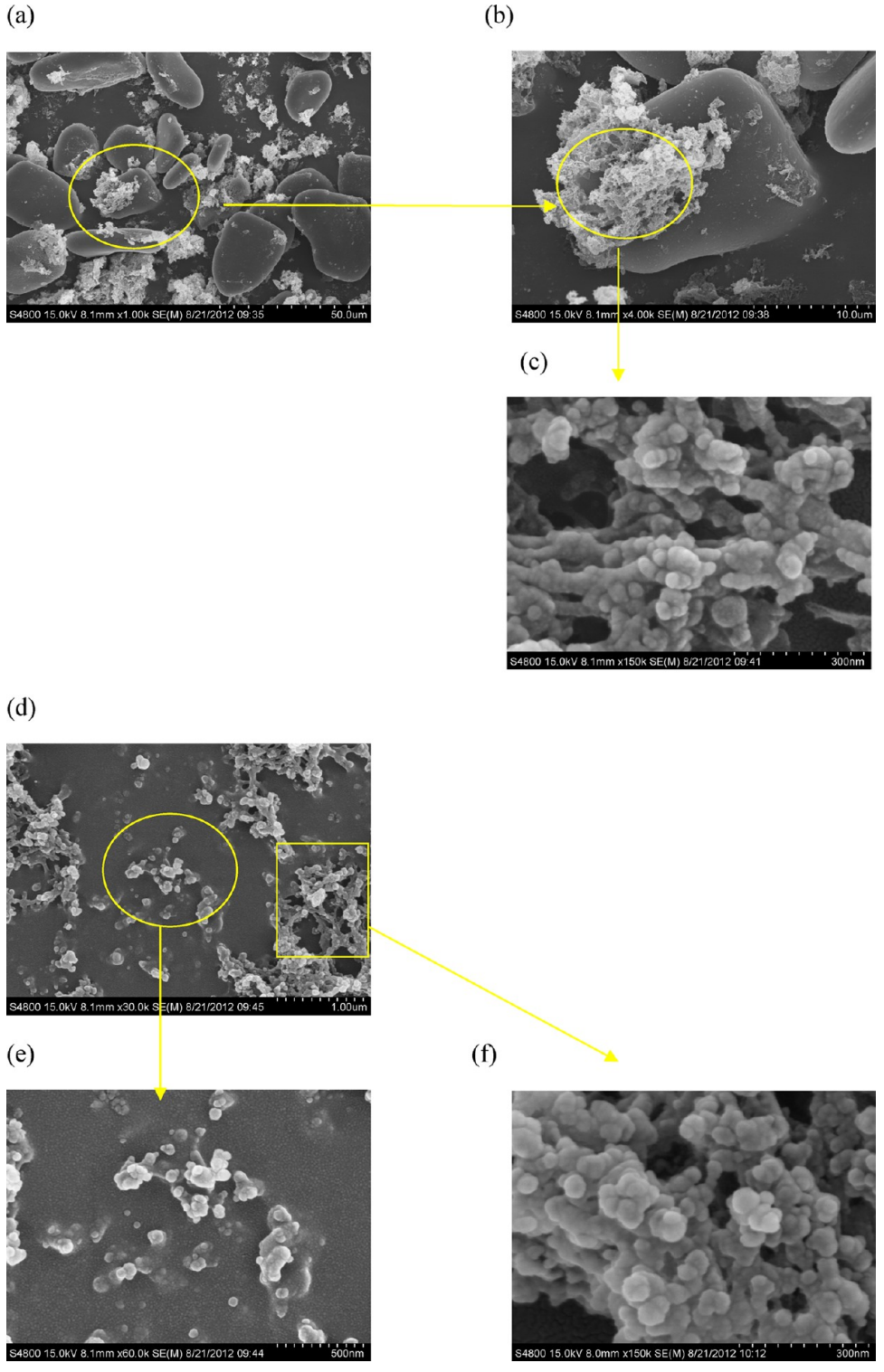

Figure 3. SEM images of (a–c) blended and (d–f) media-milled yam.

concentrations (1.7–7.8125 μg/mL), the viability was retained around 100% when the addition level was no less than 15.625 μg/mL. We did extend the experiments with higher doses (up to 2000 μg/mL), and the cell viability (data not shown) was not significantly altered. Media-milled yam did not inhibit the viability of WS1 cells, but slightly increased the viability when the addition level was greater than 15.625 μg/mL. The data illustrated that both blended and media-milled yam did not exhibit toxicity to WS1 cells. This was similar to the media-milled goji on IEC-6 and Caco-2 cells.[20] The presence of nanoparticles in media-milled yam did not exhibit any strange effect on cell viability.

Collagen Secretion. As reported in the literature, ascorbate has been shown to stimulate collagen synthesis in cultured human dermal fibroblasts through prolyl hydroxylation[38] and the gene expression of collagen without changing the rate of intracellular degradation.[39] Both ascorbate and diosgenin (at 5 and 10 μg/mL) enhanced the collagen secreted by WS1

Table 1. Proximate Composition[a−c] of Yam Samples

	composition (% db)				
				dietary fiber	
sample[d]	ash	crude protein	crude fat	insoluble	soluble
starting material	4.73 ± 0.03 ab	8.24 ± 0.07 a	0.24 ± 0.02 a	5.13 ± 0.02 a	0.77 ± 0.06 c
BY	4.81 ± 0.01 a	7.96 ± 0.06 b	0.23 ± 0.01 a	4.98 ± 0.03 b	1.03 ± 0.02 b
MY	4.67 ± 0.03 b	7.56 ± 0.06 c	0.23 ± 0.02 a	5.05 ± 0.04 ab	2.08 ± 0.04 a

[a]All results are presented on the basis of dry weight. [b]Mean ± standard error (n = 3). [c]Different lowercase letters in the same column indicate the values are significantly different at $p < 0.05$ analyzed by Duncan's multiple-range test. [d]BY = blended yam; MY = media-milled yam.

Table 2. Steroidal Saponin Contents of Yam Samples[a−c]

sample	diosgenin (μg/g)	stigmasterol[d] (μg/g)	β-sitosterol (μg/g)
starting material	37.44 ± 1.48 e	ND	22.11 ± 0.59 d
blended yam	49.65 ± 0.80 d	ND	25.48 ± 1.78 d
blended yam supernatant	18.49 ± 1.08 f	ND	ND
blended yam precipitate	50.90 ± 2.04 d	ND	27.33 ± 0.57 d
media-milled yam	128.96 ± 2.60 b	33.86 ± 0.65 b	62.89 ± 2.11 b
media-milled yam supernatant	65.88 ± 2.03 c	22.71 ± 1.59 c	41.25 ± 1.75 c
media-milled yam precipitate	176.38 ± 2.99 a	47.06 ± 1.55 a	75.06 ± 3.40 a

[a]All data are expressed per gram of dry solid. [b]Mean ± standard error (n = 3). [c]Different lowercase letters in the same column indicate the values are significantly different at $p < 0.05$ analyzed by Duncan's multiple-range test. [d]ND = not detectable.

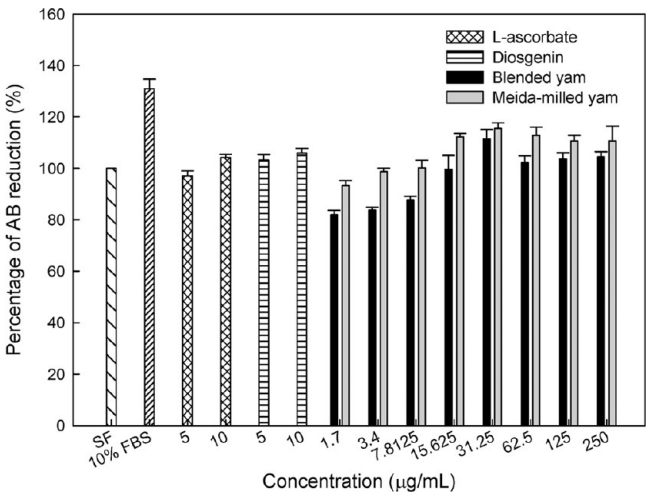

Figure 4. Cell viability of the human fetal skin fibroblast cell line (WS1) treated with L-ascorbate, diosgenin, and blended and media-milled yam for 24 h (SF = serum-free DMEM; 10% FBS = DMEM supplemented with 10% FBS).

(Figure 5a). There was about 45 μg/mL collagen from WS1 when ascorbate or diosgenin was added at 10 μg/mL. Increasing the addition level of blended or media-milled yam resulted in an increase in collagen secretion. Nevertheless, media-milled yam always resulted in more collagen than blended yam at the same addition levels. Adding 31.25 μg/mL blended yam resulted in more than 45 μg/mL collagen secretion, close to that by ascorbate or diosgenin at 10 μg/mL. Nevertheless, a lower addition level (15.63 μg/mL) of media-milled yam induced similar collagen secretion. When the addition level of the media-milled yam was no less than 31.25 μg/mL, the collagen secretion was greater than 50 μg/mL. Estimating from the data in Table 2, the concentrations of diosgenin in 31.25 mg/mL in blended and media-milled yam were 0.58 and 2.06 μg/mL, respectively. The data illustrated that diosgenin did enhance collagen secretion by WS1, and size reduction resulted in more enhancements of collagen secretion.

The data showed similar phenomena on the secretion of hTGF-β (Figure 5b). Ascorbate exhibited greater enhancement of secretion of hTGF-β than diosgenin. The highest addition level of blended yam only reached 1.6 ng/mL hTGF-β secretion, which was close to that by 5 μg/mL L-ascorbate. Nevertheless, 62.5 μg/mL media-milled yam resulted in about 2.3 ng/mL hTGF-β secretion, which was greater than that by 10 μg/mL L-ascorbate. Ascorbate and diosgenin exhibited similar effects on secretion of hEGF (Figure 5c). Blended yam resulted in the highest hEGF secretion at 27 pg/mL, which was close to that by 10 μg/mL ascorbate or diosgenin. Nevertheless, 3.4−15.63 μg/mL media-milled yam resulted in secretion of hEGF greater than 30 pg/mL. Again, media-milled yam exhibited more enhancements of hEGF secretion than blended yam. The growth factor (hTGF-β) has been shown to act on human dermal fibroblasts at the pretranslational level by stimulating the accumulation of fibronection and type I procollagen mRNAs.[40] hEGF increases the numbers of fibroblast cells by stimulating proliferation and migration of epithelial cells and by increasing synthesis of amino acids, which results in an increase in total collagen production.[41] Thus, the enhancement of hTGF-β and hEGF resulted in more collagen secretion illustrated in Figure 5a.

MMP-1 is one of the collagenases and is capable of hydrolyzing collagen I. An increase in MMP-1 results in an increase in hydrolysis of collagen and thus a decrease in the content of collagen. Ascorbate did not significantly affect the secretion of MMP-1 (Figure 6). Nevertheless, diosgenin did result in a decrease in the secretion of MMP-1. The structure and functionality of diosgenin are similar to those of estrogen (17-β estradiol), which is a precursor of sex hormones. Thus, diosgenin has been considered to be beneficial to the aging of skin. Kanda and Watanabe[42] mentioned that estrogen could enhance the production of TGF-β1 in fibroblasts and induce fibroblasts to proliferate and produce collagen. Estrogen (17-β estradiol) reduces expression of MMP-8 and MMP-13 in ovariectomized rats, which inhibits collagenolysis and restores the collagen content in the wound bed.[43] Blended yam exhibited an effect similar to that of ascorbate, even with a high addition level. The secretion of MMP-1 was decreased when the addition level of media-milled yam was increased. When 62.5 μg/mL media-milled yam (consisting of 4.12 μg of diosgenin) was added, MMP-1 secretion was similar to that induced by 10 μg/mL diosgenin. The decrease in MMP-1 secretion resulted in an increase in collagen secretion. It

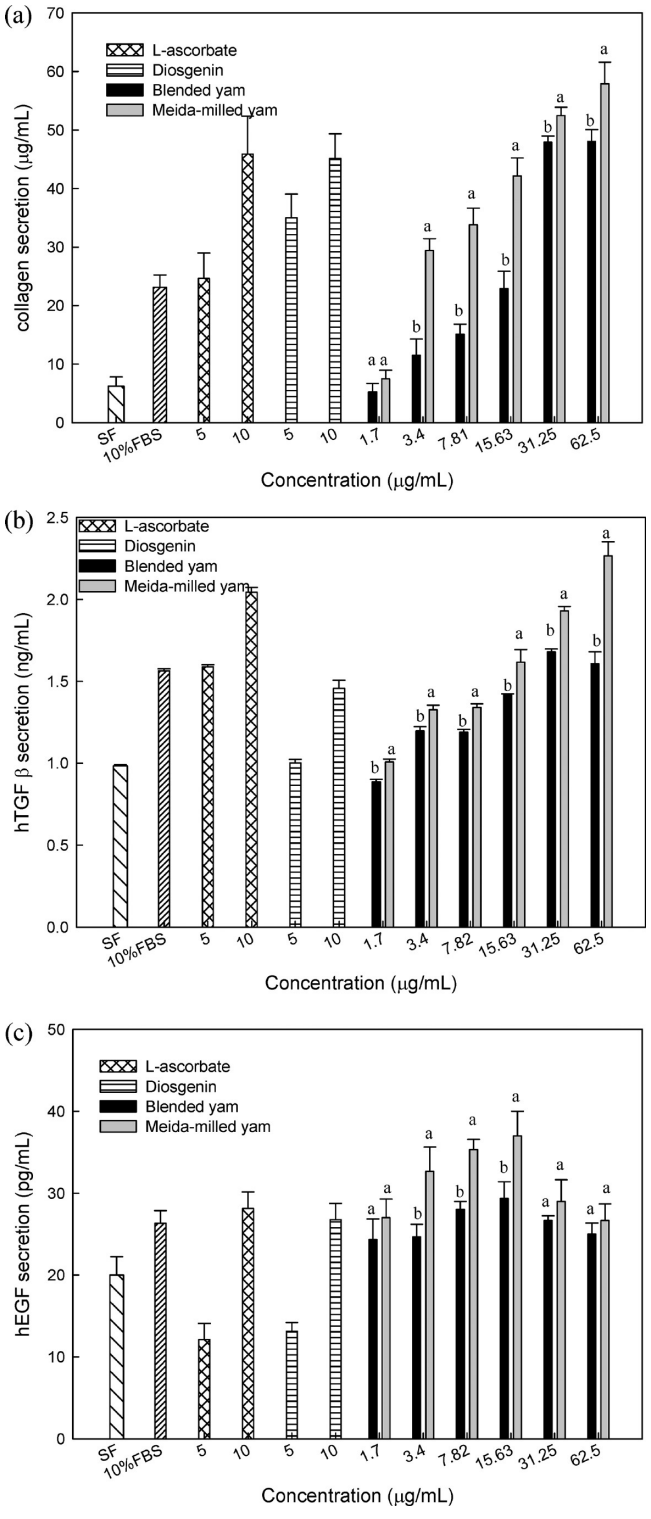

Figure 5. Collagen secretion (a), hTGFβ secretion (b), and hEGF secretion (c) of WS1 cells treated with L-ascorbate, diosgenin, and blended and media-milled yam for 24 h (SF = serum-free DMEM; 10% FBS = DMEM supplemented with 10% FBS; different lowercase letters indicate the values are significantly different between blended and media-milled yam at the same concentration at $p < 0.05$ analyzed by Duncan's multiple-range test).

appeared that size reduction did enhance the influence of yam on MMP-1 secretion.

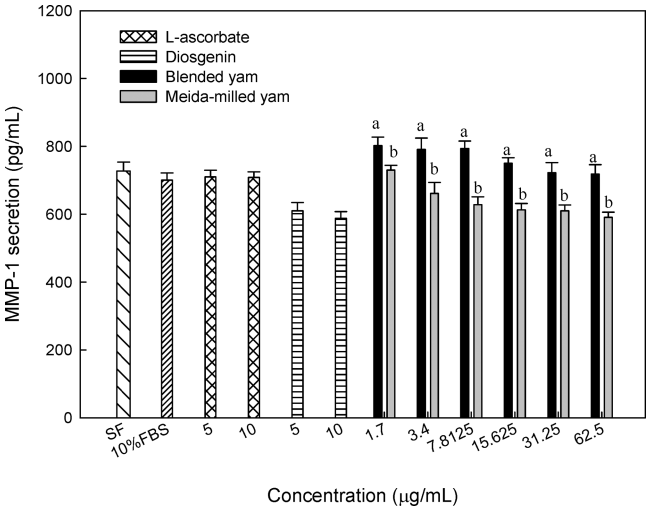

Figure 6. MMP-1 secretion of WS1 cells treated with L-ascorbate, diosgenin, and blended and media-milled yam for 24 h (SF = serum-free DMEM; 10% FBS = DMEM supplemented with 10% FBS; different lowercase letters indicate the values are significantly different between blended and media-milled yam at the same concentration at $p < 0.05$ analyzed by Duncan's multiple-range test).

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Notes

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ABBREVIATIONS USED

AB assay, AlamarBlue assay; ANOVA, analysis of variance; AOAC, Association of Official Analytical Chemists; Caco-2, human colorectal adenocarcinoma; DMEM, Dulbecco's modified Eagle's medium; EGF, epidermal growth factor; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; LC_{50}, lethal concentration 50; MMP, metalloproteinase; nm, nanometer; OD, optical density; SAS, statistical analysis system; SEM, scanning electron microscopy; TGF-β, transforming growth factor β; UV, ultraviolet; YTZ, yttria-stabilized tetragonal zirconia; μg, microgram; μm, micrometer

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