J. Cosmet. Sci., **68,** 271–283 (July/August 2017)

The antiwrinkle and antimelanogenic effects of the nonedible part of *Sorghum bicolor* (L.) Moench and their augmentation by fermentation

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Accepted for publication July 18, 2017.

Synopsis

Sorghum [*Sorghum bicolor* (L.) Moench] is a major cereal crop. Despite the wide cultivation of sorghum, its stalks are used as hay and silage. The plant has numerous bioactive compounds including cosmeceutical ingredients. Thus, we investigated the antimelanogenic and SSE that is prepared from the stalk of *Sorghum bicolor* L. (SSE) after ethanol (EtOH) extraction. Based on the antioxidant capacity, antityrosinase activity, and suppression of the protein expression levels of matrix metalloproteinase (MMP)-1, -2, and -3 in human neonatal foreskin HDF-N cells, a 50% EtOH extraction of SSEs showed antimelanogenic and antiwrinkle potential. To enrich the cosmeceutical potential of SSE, a fermentation process was applied to SSE with the use of the fungus *Aspergillus oryzae* NK (*f*SSE). On additional fermentation, the cosmeceutical potential of SSE increased with further enhancement of antityrosinase activity and suppression of MMP-1, -2, and -3 protein expression. SSE contains *p*-coumaric acid, and its level was enriched by the fermentation process. Collectively, SSE and its fermented product can serve as good ingredients in new cosmeceutical compounds.

INTRODUCTION

In modern society, skin whitening and the reduced appearance of wrinkles are effective methods for maintaining a youthful appearance, even in middle-aged or elderly people (1–3). Overexposure to the sun, especially ultraviolet radiation (UV) and normal aging may result in some undesirable effects on skin health, such as a wrinkle formation and abnormal pigmentation (4,5). Numerous environmental pollutants caused by industrialization and urbanization can impair skin health and accelerate

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skin aging (6). Extrinsic skin aging is caused by environmental factors and results in coarse wrinkles and unevenly distributed pigmentation (7,8). Intrinsic aging is associated with skin damage caused by endogenous reactive oxygen species (ROS). It has been suggested that both aging processes involve oxidative stress and an inflammatory response in the skin (8,9). In addition to their clinical uses, such as the treatment of pigmentary disorders, skin whitening compounds are used in cosmetics to obtain a lighter skin appearance (2). Because having a youthful appearance may affect mental well-being, body image, and quality of life, skin-care and antiaging cosmetics are the center of attention in different societies in countries around the world, including Asia (10,11).

Skin color is determined by a combination of four biochromes, including oxyhaemoglobin (red), reduced haemoglobin (blue), carotenoids (yellow), and melanin (orange-black) (3). Melanin is produced in the melanosomes of melanocytes and serves as a shield to protect DNA against damage caused by UV that induces ROS to act as a signal for the proliferation of melanocytes and melanogenesis. However, excessive accumulation of melanin and/or melanosome darkens skin color (12). Melanin production is regulated by a glycoprotein tyrosinase (13). Transcription of tyrosinase is controlled by microphthalmia transcription factor and/or alpha-melanocyte-stimulating hormone (14). Thus, skin depigmentation can be achieved by interfering before, during, or after melanin synthesis. In addititon, the structural integrity of the skin is maintained by extracellular matrix, such as Type I collagen, however, collagen breakdown is prevalent in photo-aged skin (5). Matrix metalloproteinases (MMPs) are zinc-containing endopeptidases that play a critical role in the degradation of collagens (15). The expression of MMPs is primarily regulated at the transcriptional level, but aberrant activation of transcription factors, such as the nuclear factor kappa B (NF-κB), activator protein-1, signal transducers and activators of transcription, and the Smad family of proteins, causes robust production of specific MMPs (16). Excessive degradation of collagens by MMPs may cause the destruction of healthy skin architecture and result in the formation of wrinkles associated with aging. In a large variety of whitening products, the use of different natural whitening agents is noticeable because of serious safety concerns associated with corticosteroids, tretinoin, and hydroquinone (2,3). Among botanic ingredients, one of the active ingredients from plants is an arbutin isolated from *uva ursi* bearberry (3). Numerous efforts aimed at preventing skin aging or obtaining skin whitening without safety concerns are underway.

Sorghum [*Sorghum bicolor* (L.) Moench] is a major cereal crop which, along with rice, maize, and wheat, is cultivated all over the world. Sorghum is used in various ways, including as food, teas, and beer, and its extracts are used in traditional medicine (17). However, unlike the grain, the byproducts of sorghum, such as the stalk, are not actively used. Leaves and stems remaining after grain harvest are used as hay and silage. Recent studies have shown that the stalk of sorghum has antioxidant, anti-inflammatory, and antianemic properties (18), but its beneficial effect on skin whitening and its skin antiaging potential have not been studied. The development of safe yet effective melanogenesis inhibitors is one of the challenges of dermatological research and the cosmetic industry. Thus, we investigated the antiwrinkle and antimelanogenesis potentials of an EtOH extract from Sorghum [*Sorghum bicolor* (L.) Moench] stalk and its fermented Sorghum stalk. We evaluated its *in vitro* antioxidant capacity, antityrosinase activity, and ability to suppress MMP-1, -2, and -3.

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MATERIAL AND METHODS

CHEMICALS AND REAGENTS

Water and acetonitrile were high-performance liquid chromatography (HPLC) grade and obtained from Honeywell Burdick & Jackson (Morris Plains, NJ). Arbutin, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®), 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), β-phycoerythrin (β-PE), mushroom tyrosinase, L-tyrosine, *p*-coumaric acid, and tumor necrosis factor-α (TNF-α) were purchased from Sigma (St. Louis, MO). Unless indicated otherwise, all other chemicals were obtained from Sigma. All antibodies were obtained from Cell Signaling Technologies (Beverly, MA).

CELL CULTURE

NIH-3T3 cells and a melanoma cell line (B16F10) were obtained from the Korean cell line bank (Seoul, Korea) and were maintained in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY) containing 10% fetal bovine serum (Gibco) and 1% penicillin (Gibco). Melanoma and fibroblast cells were cultured at 37°C in a humidified atmosphere with 5% CO_2. HDF-N cell is a primary human neonatal foreskin cell line and widely used for the wrinkle-repair efficacy test (19). HDF-N cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and were maintained in FGM-2 Bullet Kits (Lonza, Switzerland) with 1% penicillin (Gibco) at 37°C in a humidified atmosphere with 5% CO_2. For the fermentation of *Sorghum bicolor* L. stalk (SSE), *Aspergillus oryzae (A. oryzae)* NK, a widely used filamentous fungi in the fermentation process containing abundant digestive enzymes (20), was obtained from Seoul Pharmaceutical (Seoul, Korea) and was cultured at 37°C in Potato Dextrose Broth (BD Biosciences, San Jose, CA) which is used for cultivating yeasts and molds in a shaking incubator.

PREPARATION OF THE *SORGHUM BICOLOR* L. STALK EXTRACT AND ITS FERMENTATION

Sorghum bicolor L. Moench stalks were purchased from Danjoungbio (WonJoo, Korea), cut into pieces before their use, and dried in the shade at room temperature. The ethanolic extracts of SSE were extracted for 24 h at room temperature using various concentrations of ethanol ranging from 0 to 100%. Each EtOH extract was then lyophilized and stored for use in subsequent experiments. It has been suggested that the suppressive effect of compounds or extracts on MMP-1 protein expression is a good indicator of promising candidates (21). NIH-3T3 cells were treated for 24 h with extracts from different concentrations of EtOH, and the cell lysates were then subjected to immunoblotting. Based on the suppression of MMP-1 expression (Figure 1), a 50% ethanol extract of SSE was chosen. After the addition of 0.5% *A. oryzae* NK in 50% SSE, fermentation was carried out at 37°C for 48 h using spinning. The fermented SSE was lyophilized and used in subsequent experiments after dissolving in dimethyl sulfoxide (DMSO) at the appropriate concentration.

IMMUNOBLOT ANALYSIS

Protein extraction and immunoblot were performed as previously described (22). Briefly, cells were washed twice with cold Dulbecco's phosphate-buffered saline and then homogenized

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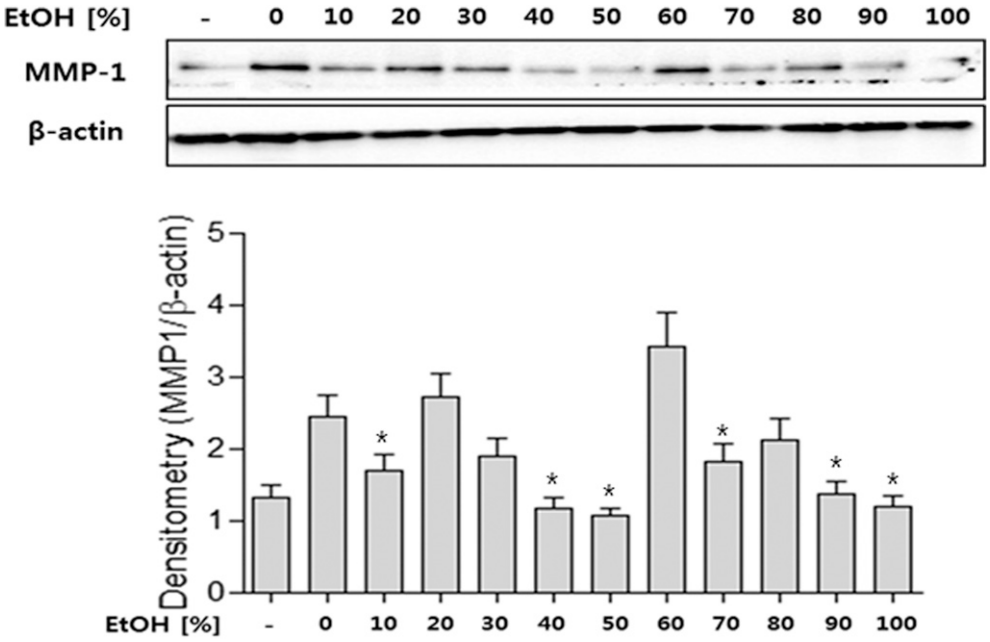

Figure 1. Effect of 50% ethanol extracted SSE on the expression levels of MMP-1 protein. NIH-3T3 cells were exposed to 50 μg/ml extracts of *S. bicolor* L. stalk produced using different concentrations of EtOH. Equal amount of cell lysate were subjected to immunoblotting. The level of MMP-1 protein is expressed relative to the level of β-actin. Data are the mean ± SEM ($n = 3$). * indicate -, control similarity. SSE; 50% ethanol extract of *S. bicolor* L. stalk, *f*SSE; *A. oryzae* NK–fermented form of SSE, MMP; matrix metalloproteinase.

in the presence of radio immunoprecipitation assay (RIPA) buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), including protease/phosphatase inhibitor cocktails; Sigma). Equal amounts of protein (30 μg) were electrophoresed on 12% SDS-polyacrylamide gels and transferred to a nitrocellulose membrane. Binding of each specific antibody was visualized using the enhanced chemiluminescence method (Amersham Biosciences, Pittsburgh, PA). Equal loading of samples was confirmed by reprobing the membranes with anti-β-actin antibody. The signals were detected by UV transilluminators according to the manufacturer's specifications (DAIHAN Scientific Co, Seoul, Korea).

The band densities from immunoblots were analyzed using Multi Gauge Ver. 3.0 software (Fujifilm, Tokyo, Japan).

CELL CYTOTOXICITY ASSAY

To evaluate the cytotoxicity of SSE and *f*SSE, a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was performed as described previously with slight modifications (23). Briefly, HDF-N cells (5×10^3 cells/well) were seeded into 96-well plates and incubated overnight. The cells were then treated with various doses of samples for 24 h. Ten microliters of 5 mg/ml MTT solution were added to each well, and the cells were incubated for 4 h. The formazan crystals were dissolved in DMSO, and the absorbance at 540 nm was determined using a Multireader (TECAN, Infinite 200, Zürich, Switzerland).

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To exclude the interference of SSE and *f*SSE in the measurements, wells containing each test agent alone were incubated and reacted with MTT. Absorbance values were normalized to the values obtained for the untreated cells to determine the percentage of survival.

ANTIOXIDANT SCAVENGING ACTIVITY

The oxygen radical absorbance capacity (ORAC) assay, which measures scavenging activity against peroxy radicals induced by AAPH, was performed as described in a previous report to determine the antioxidant capacities of SSE and *f*SSE (24). Fluorescein (FL) was used as the fluorescent probe since the loss of FL fluorescence is an indication of the extent of damage resulting from its reaction with the peroxy radical. Briefly, 2 μl of sample or Trolox was incubated with 0.2 mM of β-PE and 200 mM of AAPH in a total volume of 200 μl. To determine decreases in the amount of fluorescence, fluorescence was measured at two-minute intervals for 60 min at 37°C. All ORAC analyses were performed on a Synergy HT plate reader at 37°C with an excitation wave length of 535 nm and an emission wavelength of 590 nm. The protective effect of an antioxidant was measured by comparing with a known antioxidant, Trolox, a water-soluble analog of the antioxidative compound, vitamin E. The area under the fluorescence decay curve (AUC) for FL was calculated as follows:

$$\mathrm{AUC}\left(\text{Area under the curve}\right) = 1 + f_1/f_0 + f_2/f_0 + f_3/f_0 + \cdots + f_{19}/f_0 + f_{20}/f_0$$

ORAC value:

$$\left[\left(\mathrm{AUC}_{\text{sample}} - \mathrm{AUC}_{\text{blank}}\right)/\left(\mathrm{AUC}_{\text{Trolox}} - \mathrm{AUC}_{\text{blank}}\right)\right] \times \left(\text{molarity of Trolox}/\text{molarity of sample}\right)$$

Final ORAC values were expressed as mean ± SEM.

DETERMINATION OF TYROSINASE ACTIVITY

Tyrosinase activity was measured using a colorimetric method. Samples were dissolved in DMSO. Each well of the assay plate contained 10 μl of sample with 20 μl of mushroom tyrosinase (1,000 U/ml), and 170 μl of assay mixture (1 mM L-tyrosine, 50 mM phosphate buffer (PH 6.5), DW= 10:10:9). Reactions were incubated at 25°C for 20 minutes and the absorbance was then measured at 490 nm. Each sample was accompanied by a blank sample that contained all the components except mushroom tyrosinase. The results were compared with a control consisting of DMSO in place of sample. The percentage of tyrosinase activity was calculated as follows:

$$\left[\left(\text{Sample} + \text{tyrosinse}\right) - \left(\text{sample alone}\right)\right]/\left(\text{tyrosinase}\right) \times 100.$$

HIGH PERFORMANCE LIQUID CHROMATOGRAM ASSAY

The Shimadzu LC-20AD HPLC system (Kyoto, Japan) was used with a PDA detector set at 309 nm.

The Skypak C_{18} (250mm × 4.6 mm) was used and temperature was set at 40°C. The gradient for elution consisted of water with 0.5% acetic acid (A) and acetonitrile (B). The

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mobile phase was used under binary linear gradient conditions as follows: 0–15 min, 10–30% B; 15–30 min, 30–60% B; 30–35 min, 60–100% B; 35–40 min, 100% B; 40–41 min, 100–10% B; 41–55 min 10% B. The flow rate was 1.0 ml/min and the injection volume was 10 µl. SSE (10 mg/ml), *f*SSE 10 mg/ml), and *p*- coumaric acid (1,000 µg/ml) were dissolved in methanol using an appropriate dilution. Peaks were identified by comparing their retention time and UV-vis spectra with *p*-coumaric acid and were quantitated using the corresponding *p*-coumaric acid curve.

STATISTICAL ANALYSIS

Data were expressed as means ± SEM. Statistical analyses were performed using the Sigma Plot 12.0 software program. One-way analysis of variance was used to identify significance ($p < 0.05$). When significant, specific differences between groups were identified using Tukey's multiple-comparison tests.

RESULTS

DETERMINATION OF OPTIMAL ETOH EXTRACTION OF SSE AND ITS CYTOTOXICITY

To find the optimal EtOH extraction of *S. bicolor* L. stalks containing high MMP-1 suppressing potential, each EtOH fraction of SSE (50 µg/ml) was used to treat NIH-3T3 cells for 24 h. The expression levels of MMP-1 were then determined by immunoblotting. As shown in Figure 1, a 50% EtOH extraction of *S. bicolor* L. stalk showed the highest suppression of collagen-degrading enzyme MMP-1 expression. In a subsequent experiment, SSE lyophilized from a 50% EtOH extraction of *S. bicolor* L. stalk was used. When the cytotoxicities of SSE and *f*SSE were assessed, there was no significant cytotoxicity in HDF-N cells treated with up to 100 µg/ml for 24 h (Figure 2). SSE and *f*SSE resulted in cell cytotoxicity above 200 µg/ml, but the cytotoxicity of *f*SSE was less than that of its original extract, SSE. These findings suggest that both SSE and *f*SSE could be produced as effective active ingredients with no associated cytotoxicity when used at concentrations up to 100 µg/ml.

MEASUREMENT OF SSE AND FSSE ANTIOXIDANT ACTIVITY

Oxidative-stress promotes MMP activity and melanin synthesis, which are causal factors in the induction of skin aging (15). Here, the antioxidant activities of SSE and *f*SSE were determined using an ORAC assay. As shown in Table I, both SSE and *f*SSE showed remarkable antioxidant scavenging activity. The antioxidant capacity of SSE at 50 µg/mL was similar to that of Trolox, and *f*SSE showed higher antioxidant scavenging activity even in lower concentrations compared with SSE.

TYROSINASE INHIBITORY EFFECT OF SSE AND FSSE

The antioxidant scavenging activities of SSE and *f*SSE were demonstrated by the ORAC assay (Table I). Skin pigmentation is caused by melanin production mediated by tyrosinase,

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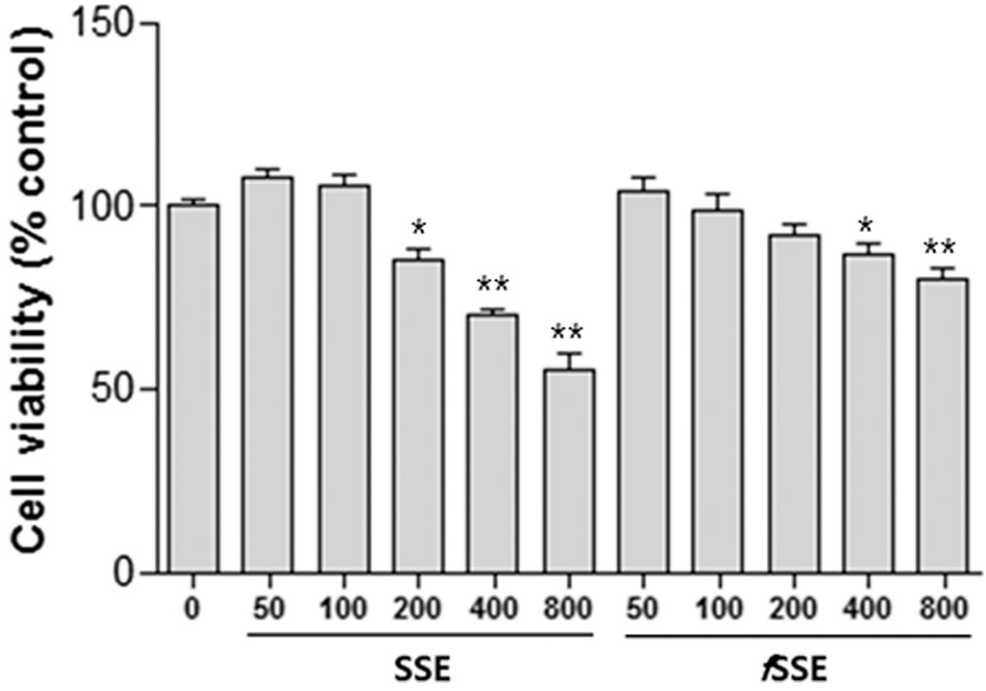

Figure 2. Cell cytotoxicities of SSE and *f*SSE. HDF-N cells were treated with different doses of SSE and *f*SSE for 24 hr. Then cell cytotoxicity was determined by MTT reduction assay. Data are the mean ± SEM ($n = 3$). * and ** indicate $p < 0.05$ and $p < 0.01$ versus untreated control. SSE; 50% ethanol extract of *S. bicolor* L. stalk, *f*SSE; *A. oryzae* NK- fermented form of SSE, MTT; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

however, its inhibition is one scheme used to inhibit the pigmentation process (12). Thus, the level of anti-tyrosinase activity, another determinant in skin whitening, was determined using purified mushroom tyrosinase. Arbutin (100 μg/ml) was used as a positive control for tyrosinase inhibition. The antityrosinase activity of SSE was evident at a dose of 50 μg/ml, but *f*SSE showed inhibitory effects at lower concentrations than SSE (Figure 3). This result suggests that the fermentation process enriched the content of active compounds involved in tyrosinase inhibition.

Table I
Antioxidant Effects of SSE and *f*SSE

μg/ml	SSE	*f*SSE
3.125	0.32 ± 0.02	0.35 ± 0.02
6.25	0.37 ± 0.01	0.55 ± 0.02
12.5	0.66 ± 0.07	0.79 ± 0.02
25	0.85 ± 003	0.98 ± 0.01
50	1.02 ± 0.03	1.09 ± 0.00
100	1.11 ± 0.01	1.12 ± 0.00
200	1.33 ± 0.03	1.29 ± 0.01

Antioxidant capacity was determined using an oxygen radical absorbance capacity (ORAC) assay. Trolox (6.25 μg/ml) was used as a positive control and set at 1.0. Data are the mean ± SEM (n=3) and expressed as an index of Trolox. SSE; 50% ethanol extract of *S. bicolor* L. stalk, *f*SSE; *A. oryzae* NK- fermented form of SSE.

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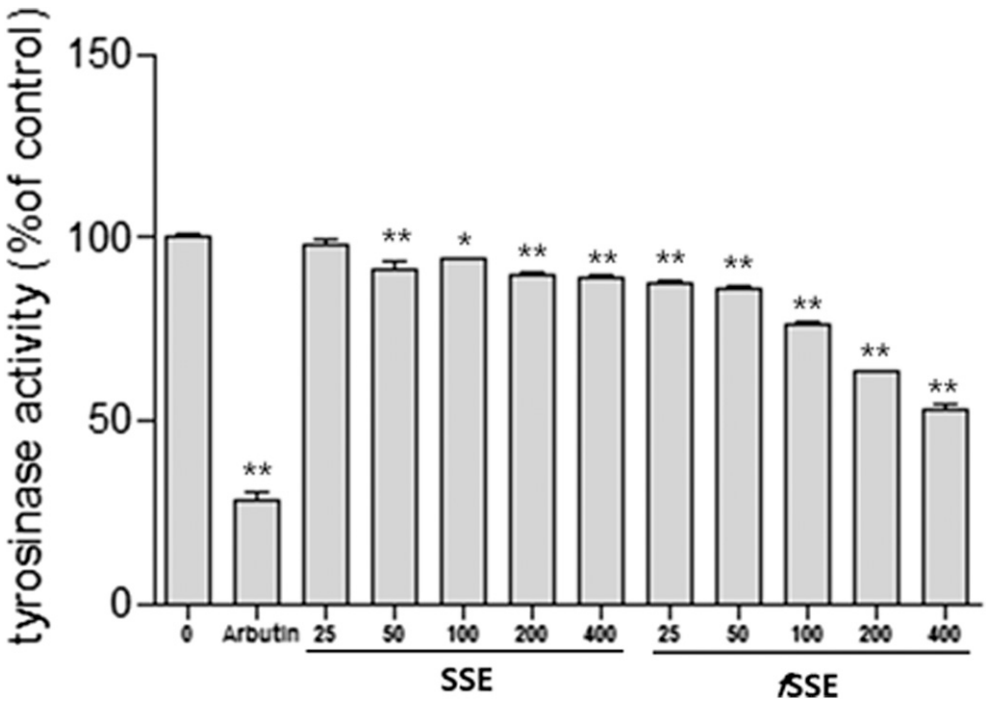

Figure 3. Tyrosinase inhibitory effect of SSE and *f*SSE. Antityrosinase activity was determined using a colorimetric method using purified mushroom tyrosinase and L-tyrosine at the indicated doses of SSE and *f*SSE. Data are % of untreated control as mean ± SEM ($n = 3$). * and ** indicate $p < 0.05$ and $p < 0.01$ versus untreated control, respectively. SSE; 50% ethanol extract of *S. bicolor* L. stalk, *f*SSE; *A. oryzae* NK-fermented form of SSE.

SUPPRESSION OF MMP-1, -2, AND -3 BY SSE AND FSSE

The suppressive effects of SSE and *f*SSE on MMP-1, -2, and -3 protein expression levels were determined by immunoblotting. Here, TNF-α (10 ng/ml) was used as an inducer of MMP expression in HDF-N cells (16,17). As shown in Figure 4, the protein expression levels of MMP-1, -2, and -3 were significantly increased in the presence of TNF-α. SSE at concentrations more than 100 μg/ml significantly suppressed the expression of MMP-1, -2, and -3. However, *f*SSE was more potent than SSE because *f*SSE showed suppressive effects beginning at 50 μg/ml. This result implies that fermentation of SSE with *A. oryzae* NK increased the components associated with inhibiting the protein expression of MMP-1, -2, and -3. This result suggests that SSE and *f*SSE could have potential as antiwrinkle agents for use in cosmeceutical products.

IDENTIFICATION OF *P*-COURMARIC ACID IN SSE AND FSSE USING HPLC AND ITS SUPPRESSIVE EFFECT ON EXPRESSION OF MMP-1 PROTEIN

To identify components increased in *f*SSE relative to SSE, HPLC was performed on SSE and *f*SSE. As shown in Figure 5A, the amount of *p*-coumaric acid in *f*SSE was 1.5-times higher than that in SSE. When the suppressive effect of *p*-coumaric acid on expression of MMP-1

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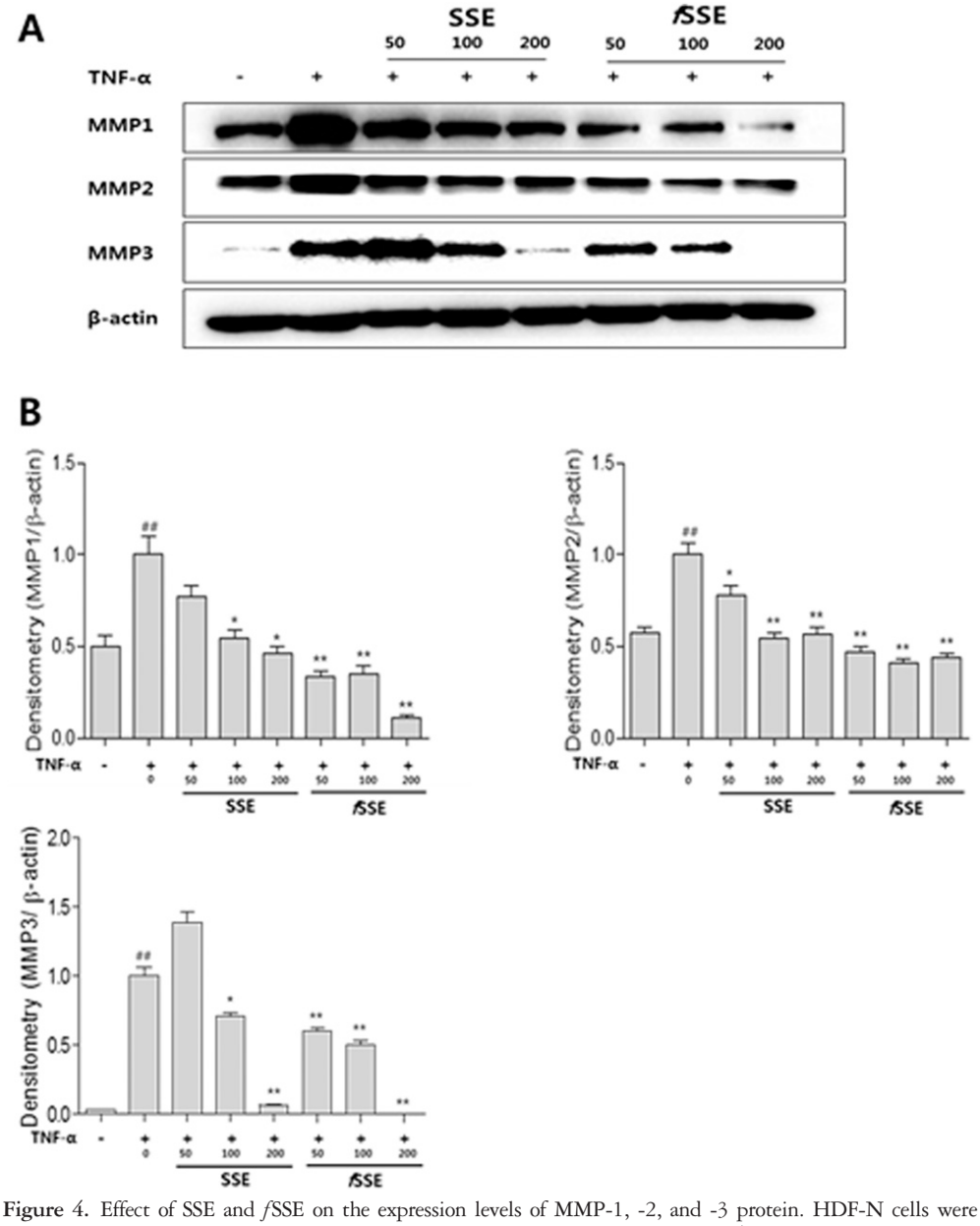

Figure 4. Effect of SSE and *f*SSE on the expression levels of MMP-1, -2, and -3 protein. HDF-N cells were treated with different doses of SSE and *f*SSE in the presence TNF-α (10 ng/ml) for 24 h. Then, equal amounts of cell lysate were subjected to immunoblotting ($n = 3$). (A) Representative image of immunoblots. (B) Densitometric analysis of immunoblots. The expression level of protein is expressed relative to the level of β-actin. * and # indicate $p < 0.05$ versus untreated control and TNF-α treated group, respectively. SSE; 50% ethanol extract of *S. bicolor* L. stalk, *f*SSE; *A. oryzae* NK- fermented form of SSE, MMP; matrix metalloproteinase, TNF-α; tumor necrosis factor-α.

protein after induction by TNF-α was determined by immunoblotting, *p*-coumaric acid was shown to be highly potent in the suppression of MMP-1 expression (Figure 5B). This result implies that *p*-coumaric acid has an inhibitory effect on MMP-1 protein expression and

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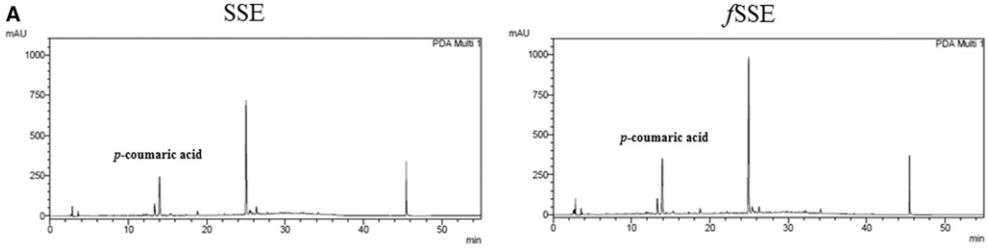

	Content of compounds [mg/g]	
Compound	SSE	*f*SSE
p-coumaric acid	2.65 ± 0.09	3.82 ± 0.12

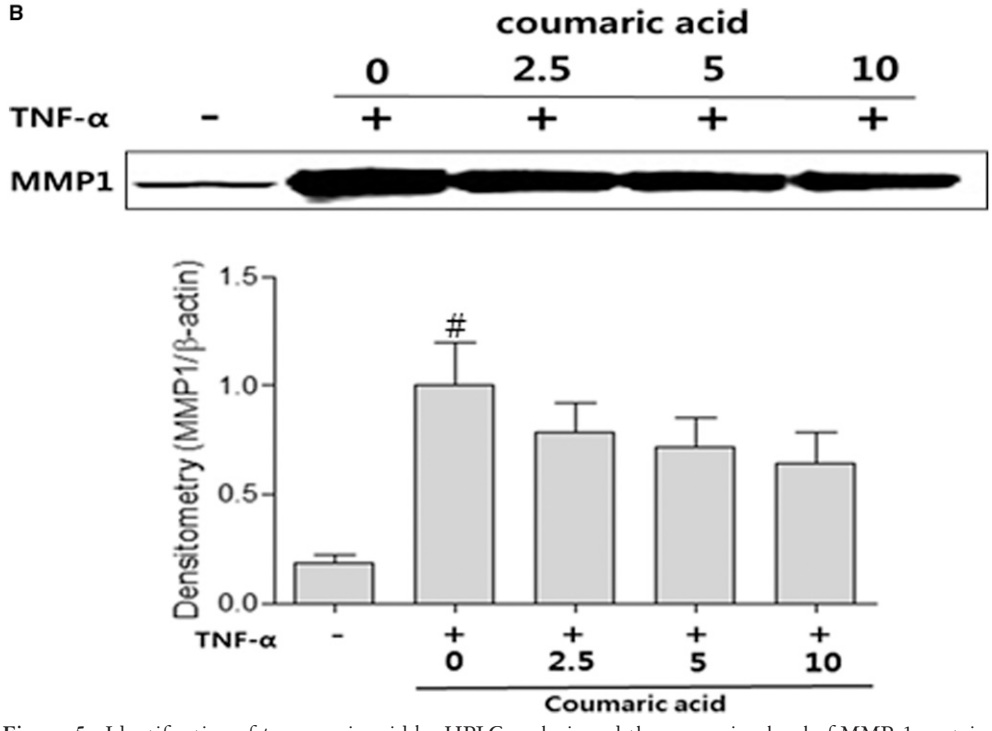

Figure 5. Identification of *p*-coumaric acid by HPLC analysis and the expression level of MMP-1 protein. (A) The amounts of *p*-coumaric acid in SSE and *f*SSE were determined by HPLC ($n = 3$). Commercial *p*-coumaric acid was used as a reference material. (B) HDF-N cells were treated with different doses of *p*-coumaric acid in the presence TNF-α (10 ng/ml) for 24 h. Then, equal amounts of cell lysate were subjected to immunoblotting ($n = 3$). The level of MMP-1protein is expressed relative to that of β-actin. * and # indicate $p < 0.05$ versus untreated control and TNF-α treated groups, respectively. SSE; 50% ethanol extract of *S. bicolor* L, MMP; matrix metalloproteinase, TNF-α; tumor necrosis factor-α.

augments the suppressive effect of *f*SSE on MMP-1. Consequently, the enhanced activities of *f*SSE may be associated with increases in *p*-coumaric acid resulting from fermentation. However, we cannot exclude the possibility that other components may be involved in the inhibitory effect of *f*SSE, either alone or a combinatorial manner.

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DISCUSSION

In this study, an EtOH extract of *S. bicolor* L stalks showed antioxidant, antityrosinase, and antiwrinkle activities. These activities of SSE were augmented after fermentation with *A. oryzae* NK. In *f*SSE, the amount of *p*-coumaric acid was increased after fermentation of SSE, and this compound may be, at least in part, associated with the improved actions of *f*SSE with respect to antiwrinkle activity.

Skin aging is a major issue today, involving processes of photo-aging due to industrial pollution and global warming (3). Considering the impacts of having a healthy face and skin in maintaining quality of life, remedies for preventing or suppressing both melanogenesis (through the inhibition of tyrosinase activity) and wrinkle formation mediated by MMPs are of great interest. *Sorghum bicolor* L. Moench is rich in phytochemicals, such as tannins, phenolic acids, anthocyanins, phytosterols, and policosanols, known to significantly affect human health, (17).

Compared with sorghum grain, the stalk constitutes a much greater proportion of the plant mass and may provide a more cost-effective way of obtaining large quantities of cosmeceutical ingredients. With respect to this, we found that SSE had antioxidant capacity, could suppress both tyrosinase activity (Figure 3) and MMP-1, -2, and -3 protein expression levels (Figure 4), and had additional antioxidant capacity (Table I). To enhance the cosmeceutical effect of SSE, the effect of fermentation of SSE was tested. *f*SSE showed increased antityrosinase activity (Figure 3) and suppressed the protein expression levels of MMP-1, -2, and -3 (Figure 4).

Previous reports have suggested that *p*-coumaric acid possesses antityrosinase and antimelanogenesis activities because of its similarity to the chemical structure of tyrosine (25,26). SSE containing *p*-coumaric acid (Figure 5A) showed significant tyrosinase-inhibitory activity; however, its reduction potential was relatively less than expected (Figure 3). This low potential of SSE as a tyrosinase inhibitor may be associated with the use of mushroom tyrosinase because the tyrosinase-inhibitory activity of *p*-coumaric acid is stronger against human tyrosinase than against murine or mushroom tyrosinase. At any rate, the enhanced tyrosinase-inhibitory activity of *f*SSE may reflect the increased amount of *p*-coumaric acid after fermentation (Figure 5A). This result suggests that fermentation of SSE with *A. oryzae* NK will be effective in enriching the cosmeceutical ingredients in SSE.

When we compared the compositions of SSE and *f*SSE, *p*-coumaric acid was enriched in *f*SSE and was able suppress the level of MMP-1 protein. *p*-Coumaric acid (4-hydroxycinnamic acid) is a phenolic acid found ubiquitously in plants and mushrooms in free or bound forms (27). Numerous activities, including antioxidant, anti-inflammatory, antimutagenic, antiulcer, antiplatelet, and anticancer activities, associated with *p*-coumaric acid have been reported (27). In this experiment, the suppressive effect of *p*-coumaric acid on the expression of MMP-1 protein in HDF-N cells was identified (Figure 5B). However, the elucidation of its underlying mechanisms will require more extensive work. Further identification of other active compounds involved in the suppression of MMP-1, -2, -3 expression and inhibition of tyrosinase might be valuable, and thus, additional effort will be required in future experiments.

Collectively, SSE possesses antimelanogenic and antiwrinkle activities, and these activities of SSE were further enhanced by fermentation with *A. oryzae* NK. The *p*-coumaric acid enriched by fermentation of SSE seems to be one of active compound in SSE. This finding may provide insight for the development of cosmeceutical ingredients using the nonedible part of *S. bicolor* L. Moench.

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ACKNOWLEDGMENTS

This research was supported by the Ministry of Trade, Industry & Energy (MOTIE), Korea Institute for Advancement of Technology (KIAT) through the Encouragement Program for The Industries of Economic Cooperation Region (R0000429).

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