

# A Study on the Fabrication of an Effective Natural Substance Based on *Schisandra chinensis* Extracted Fermentation

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**Purpose:** In this study, a high-efficiency *Schisandra chinensis* extract (SCE) produced by the fermentation of effective microorganisms (EM) was used as an antioxidant material in preparing cosmetic products.

**Subjects and Methods:** We conducted the study by extracting *S. chinensis* via EM fermentation to increase the efficiency. Tyrosinase inhibitory factor analysis, pH, and thermal stability were measured to verify the properties of the prepared products.

**Results:** The efficacy and whitening effects of the prepared substances were verified using tyrosinase inhibitory factor analysis. As a result, it was found that both the SCE and SCE fermentation (SCEF) exhibited high, naturally originating, antioxidation ability. In addition, the pH and thermal stability of the substances were evaluated to optimize the cosmetic fabrication conditions. In this context, as the concentration of the added extract increased, the pH value decreased. The evaluation of safety and stability indicated that the substances contained effective chemical components having antioxidant activity, suppressing skin aging, and whitening effects in a weak acid range consistent with a pH of 6.25–2.98. Furthermore, there were no safety problems with the use of the obtained products even after they had been stored for 60 days.

**Conclusion:** The SCE substance is demonstrated as a possible material for cosmetic application.

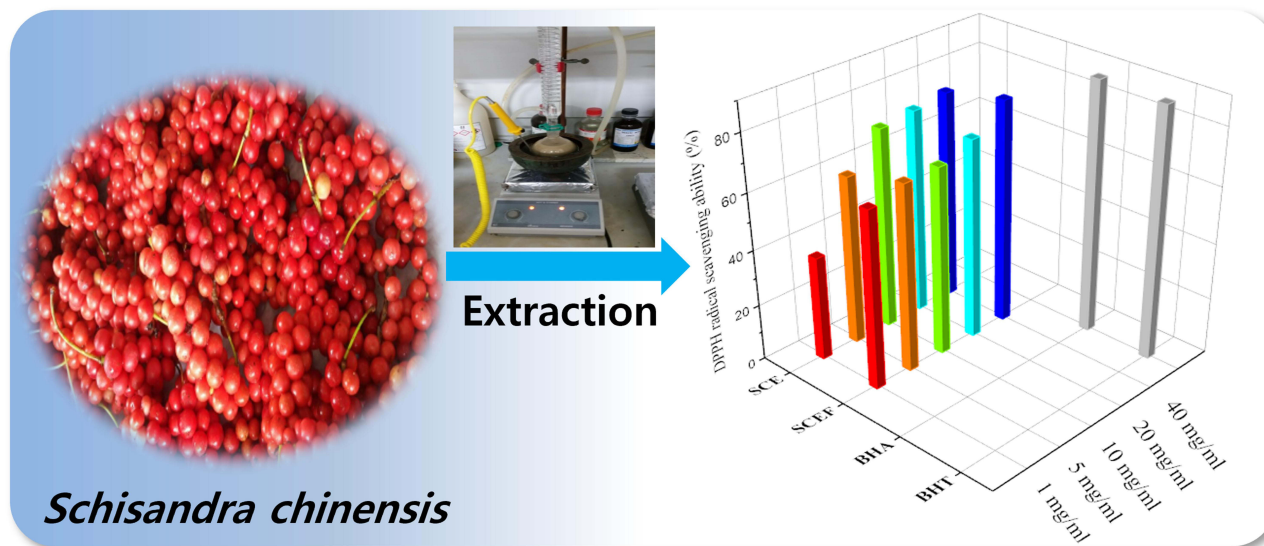
**Keywords:** *Schisandra chinensis*, effective microorganisms fermentation, antioxidation

## Introduction

In recent years, the improvement and the pursuit of high living standards have caused a lot of problems affecting people's health. Among these, cosmetic products are a particular example that needs to be carefully investigated. The use of synthetic chemicals as a main ingredient of cosmetic products leads to many negative results, such as toxicity and high cost. This is the reason why the investigation of natural products for cosmetic applications has attracted a lot of interest from many researchers. *Schisandra chinensis* (SC) is a medicinal and edible plant that has five tastes (sweet, sour, bitter, salty, and pungent),<sup>1,2</sup> and is widely used in many foods, drinks, and herbal industries, etc.<sup>3,4</sup> SC contains many bioactive compounds, such as gomisin, malic acid, and citric acid, and can be effectively used to treat cough and asthma.<sup>5</sup> In addition, it can be used in the food and cosmetic industries owing to its well-known antibacterial and antioxidant abilities. Furthermore, it has excellent heat stability and can be used in cosmetics and

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Graphical Abstract



foods that do not affect people’s health.<sup>6</sup> Fermentation refers to the process of decomposing organic material using the enzymes of microorganisms, and is derived from the Latin word *fervere*.<sup>7</sup> It has been used in various ways and in different fields, such as food, drugs, and cosmetics.<sup>7</sup> Food fermentation through the enzymatic action of microorganisms is traditionally used in manufacturing processes to improve flavor and destroy toxins, and also has the effect of promoting the biomolecules.<sup>8,9</sup> Effective microorganisms (EM) are useful microorganisms which were developed in 1982.<sup>10</sup> EM was originally developed for use in natural and organic farming. Subsequently, its applicable scope has been gradually extended and it is commonly used in Asian countries, Russia, and the USA.<sup>11</sup> Initially, the solution of EM was developed from 80 species of 10 genera in 5 families; however, it was a very complex process. Therefore, EM was then simply developed by some principal organisms, such as photosynthetic bacteria, lactic acid bacteria, fungi, yeast, and actinomycetes.<sup>12</sup> Regarding the fermentation application, it is fermented in facultative anaerobic conditions, so its synthesis products, such as vitamins and carotene pigments as powerful antioxidants to prevent the decay of organic material.<sup>13</sup> The resulting amino acids and organic acids are converted to the respective proteins and sugars and then absorbed immediately by the plant. This improves greatly the efficiency of both

the synthesis and use of plant food. EM was originally developed for use in natural, organic agriculture, but nowadays is used in a variety of fields such as construction, medical, and cosmetic industries.<sup>14–16</sup>

In this study, SC was extracted by hot water and the extract broth was made by EM fermentation to increase the efficiency. Tyrosinase inhibitory factor analysis was used to determine the efficacy and whitening effects. Besides, the pH and thermal stability were measured to verify the stability of the prepared products, then the validity of the material with regard to the antioxidant and whitening effects of SC was evaluated.

**Materials and Methods**

**Materials**

SC fruits were purchased from a farm in the Republic of Korea. SC was treated with deionized (DI) water and then dried naturally in well-ventilated shade to produce the base material. Diethylene glycol (DEG, (HOCH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>O), folin–Ciocalteu’s phenol reagent, sodium hydroxide (NaOH), and sodium citrate tribasic dihydrate (Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>) were purchased from Sigma Chemical Co. (USA). Purified DI water was used for all experiments. EM active solution, as a reagent for EM fermentation, was purchased from the EM Center, Jeonju University (Republic of Korea).

## SC Extraction

The dried SC was extracted using a reflux condenser 10 times in hot water after titrating. The extraction temperature was 80°C, consistent with the extraction time of 24 h. Impurities were removed from the SC solution by a filtering process. The filtered solution was evaporated in a rotary evaporator (N-1000SW), and then freeze-dried for one day to remove the solvent completely, leaving a solid product. The obtained product was stored in vacuum conditions during the experiments.

## Preparation of Different Extract Concentrations

To compare and analyze the extract from ordinary SC and from EM-fermented SC, different concentrations of 1, 5, 10, 20, and 40 mg·mL<sup>-1</sup> were produced. In the case of the extract from EM-fermented SC, each portion of EM-fermented liquor and sugar was maintained at 6% and 5%. The extract from EM-fermented SC was incubated at 37°C for 7 days before being used.

## Microelement Analysis

In accordance with the Food Code method,<sup>17</sup> 1.0 g of the sample was dissolved in nitric acid 100 mL with DI water at 100°C. Then, the amounts of trace elements in the sample were measured and analyzed with an Elemental Analyzer (Vario EL, Germany).

## Measurement of Polyphenol Content

The polyphenol content per gram of the sample was measured by the Folin–Denis method.<sup>18</sup> Thus, 100 µL of extract and 2 wt% Na<sub>2</sub>CO<sub>3</sub> were mixed in an EP tube. The EP tube was kept at room temperature for 2 min for the reaction. After that, 50% Folin–Ciocalteu's phenol reagent was added to the tube. The sample was put in a vortex mixer at room temperature for 30 min, and then analyzed with a UV-Vis spectrophotometer at 750 nm.

## Measurement of Flavonoids

The total content of flavonoid per gram of extract was measured by diethylene glycol colorimetry.<sup>19</sup> Thus, 100 µL of extract and 100 µL of 1.0 N NaOH were added to an EP tube and mixed using a vortex mixer. After mixing, the solution was kept at 30°C for 1.0 h for the reaction. The yield of the reaction was analyzed by a UV-Vis spectrophotometer at 420 nm.

## Measurement of Free-Radical Scavenging

The free-radical scavenging by 1,1-diphenyl-2-picrylhydrazyl (DPPH) was measured by the modified Blois method.<sup>20</sup> In this context, 0.1 M Trizma base–HCl buffer (Tris buffer, pH 7.4) and 500 mM DPPH were initially prepared with methanol. Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were selected as standards for the control experiment. Then, 100 µL of extract samples and 400 µL of Tris buffer were mixed in an EP tube, followed by the addition of 500 µL of DPPH solution. The mixture was kept in a dark room for 20 min, then analyzed by a UV-Vis spectrophotometer at 517 nm. In the control experiment, 100 µL of BHT and BHA were added instead of the extract samples. In the non-additive group, 100 µL of Tris buffer was added to the EP tube instead of the extract samples. The measurement of electron donation ability is shown as follows:<sup>21</sup>

$$EDA(\%) = \left( 100 - \frac{\text{Absorbance of additive}}{\text{Absorbance of Non-additive}} \right) \times 100 \quad (1)$$

## Measurement of Nitrite Scavenging Activity

The nitrite scavenging activity was measured using the modified method developed by Kim et al.<sup>22,23</sup> Specifically, 0.3 mL of the extract sample and 0.1 mL of 1.0 mM NaNO<sub>2</sub> solution, 0.2 M citrate buffer–HCl at pH 2.5 was mixed to obtain a final volume of 1.0 mL. The mixture was then reacted at 37°C for 1.0 h. Subsequently, it was mixed with 0.4 mL Griess reagent (30% CH<sub>3</sub>COOH solution containing sulfanilic acid (1.0 wt%): naphthylamine (1 wt%)) and 3.0 mL of CH<sub>3</sub>COOH solution (2.0 wt%). Then, the reaction took place at room temperature for 15 min.

$$\text{Absorbance scavenging} = \left( 1 - \frac{A - B}{C} \right) \times 100 \quad (2)$$

where *A* is the absorbance at 520 nm determined with test sample, *B* is the absorbance at 520 nm determined with HO instead of NaNO<sub>2</sub>, and *C* is the absorbance at 520 nm determined with H<sub>2</sub>O instead of the test sample, using a UV-Vis spectrophotometer.

## Measurement of Superoxide Dismutase-Like Activity (SODA)

Superoxide dismutase (SOD)-like activity (SODA) was measured using according to Marklund and Marklund's

method. The degree of oxidation of the pyrogallol catalyzing the reaction to convert hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is shown in SODA.<sup>24</sup> For this, 0.2 mL of sample (pH 8.5) and Tris–HCl buffer (50 mM Tris [hydroxymethyl] aminomethane, 10 mM EDTA, pH 8.5) 2.6 mL was added to 7.2 mM pyrogallol 0.2 mL and mixed using a vortex mixer. After 10 min, the reaction was stopped by adding 1.0 N HCl solution. It was measured at 420 nm using a UV-Vis spectrophotometer. SODA demonstrated the difference in absorbance between the sample addition group and the non-addition group as a percentage.

$$\text{Superoxide dismutase – like activity} = \left(1 - \frac{B}{A}\right) \times 100 \tag{3}$$

where *A* is the absorbance of standard solution without the added sample and *B* is the absorbance of standard solution with the added sample .

### Measurement of Tyrosinase Inhibitory Activity

Tyrosinase inhibitory activity was measured by a modified version of the method presented by Masamoto et al.<sup>25</sup> To measure the mushroom tyrosinase-deactivating properties in in vitro conditions, 0.3 mL of 2.5 mM 3,4 dihydroxyphenylalanine (L-DOPA), 0.05 mL of the extract sample, and 0.1 M phosphate buffer solution (pH 6.8, total volume 1.5 mL) were mixed using a vortex mixer, and then pre-incubated at 25°C. Then, 0.05 mL of mushroom tyrosinase at 1380 units·mL<sup>-1</sup> (Sigma Co., USA) was added and then mixed using a vortex mixer. After that, the reaction was performed at 25°C for 2.0 min.

$$\text{Tyrosinase inhibitory activity} = \left(100 - \frac{A - B}{A}\right) \times 100 \tag{4}$$

where *A* is the absorbance value between 0.5 and 1 min of the reaction solution without sample, measured at 475 nm by a UV-Vis spectrophotometer; and *B* is the absorbance value between 0.5 and 1.0 min of the reaction solution with sample, measured at 475 nm by a UV-Vis spectrophotometer.

### Preparation of Cream Material

The cream formulas, based on distilled water, extracted oil, and additives, were prepared as listed in Table 1. Water, additives, and oil were weighed and then heated at 80°C in a water bath. Water was slowly added and vigorously mixed with oil in a mini mixer (DS-1800; Korea). Cream A was prepared without the extracted oil. Creams B, C, D, E, and F contained 1, 5, 10, 20, and 40 mg·mL<sup>-1</sup> of SC extract (SCE), respectively. Creams G, H, I, J, and K contained 1, 5, 10, 20, and 40 mg·mL<sup>-1</sup> of SCE fermentation (SCEF).

### Assessment of Safety

The pH values were measured by a pH meter (professional meter pp-15; Germany) at 25°C for 10 min. The glass electrode was immersed in a basic buffer solution or DI water before the pH measurement.

### Assessment of Stability

To evaluate the stability according to environmental temperature, the stability of cream containing SCE or SCEF was evaluated at 4, 25, and 40°C.

**Table 1** Different Basic Cream Recipes Containing SCE and SCEF

Sample	DI Water (g)	Extract Fermentation (g)	Avocado Oil (g)	Vitamin E (g)	Emulsifier (g)
Cream A	79	0.0	6.0	6.0	9.0
Cream B	76	3.0	6.0	6.0	9.0
Cream C	76	3.0	6.0	6.0	9.0
Cream D	76	3.0	6.0	6.0	9.0
Cream E	76	3.0	6.0	6.0	9.0
Cream F	76	3.0	6.0	6.0	9.0
Cream G	76	3.0	6.0	6.0	9.0
Cream H	76	3.0	6.0	6.0	9.0
Cream I	76	3.0	6.0	6.0	9.0
Cream J	76	3.0	6.0	6.0	9.0
Cream K	76	3.0	6.0	6.0	9.0

**Note:** A is the cream without extracted oil (0.0 mg·mL<sup>-1</sup>); B is the SCE-based cream (1.0 mg·mL<sup>-1</sup>); C is the SCE-based cream (5.0 mg·mL<sup>-1</sup>); D is the SCE-based cream (10 mg·mL<sup>-1</sup>); E is the SCE-based cream (20 mg·mL<sup>-1</sup>); F is the SCE-based cream (40 mg·mL<sup>-1</sup>); G is the SCEF-based cream (1.0 mg·mL<sup>-1</sup>); H is the SCEF-based cream (5.0 mg·mL<sup>-1</sup>); I is the SCEF-based cream (10 mg·mL<sup>-1</sup>); J is the SCEF-based cream (20 mg·mL<sup>-1</sup>); and K is the SCEF-based cream (40 mg·mL<sup>-1</sup>).

**Table 2** ICP Analysis of the SCE

SCE (mg kg <sup>-1</sup> ) Element					
	1.0	5.0	10	20	40
K	23.71	149.66	315.48	660.42	1638.42
Mn	0.15	0.80	1.62	2.80	5.69
Fe	0.42	0.58	1.08	1.66	3.32
Cu	0.02	0.02	0.04	0.07	0.12
Zn	0.00	0.04	0.11	0.22	0.47
Se	0.03	0.03	0.03	0.03	0.03

## Results and Discussion

### Microelement Analysis

The results of inductively coupled plasma mass spectrometry (ICP) analysis of SCE are shown in Table 2, which indicates that 1.0 mg·mL<sup>-1</sup> of the SCE contains 23.71, 0.42, and 0.03 mg·kg<sup>-1</sup> of K, Fe, and Se, respectively. When the amount of SCE increased, the content of these trace elements increased. In this regard, the increase in K was dominant, and the increases in Mn, Fe, Cu, and Zn were not significant. The content of Se remained the same regardless of the concentration of SCE. These trace elements help the actions of many physiologically active substances both inside and outside the human body, and serve important roles, including antioxidizing and immunity activities.

### Measurement of Extract and Its Content of Flavonoids and Polyphenols

The content of the SCE was 27.91 wt% in 100 g of SC. The extraction provided the same yield when the procedure was performed in water and ethanol. However, the yield of extract was lower than that in previous papers.<sup>26,27</sup> This is due to the differences in the places where the SC was grown, as well as the conditions of culture and the method of extraction.<sup>26</sup> The polyphenol content is shown in Table 3. The extract of ordinary SCE at 1.0 mg·mL<sup>-1</sup> provided 1.53±0.02 mg·g<sup>-1</sup> of polyphenol, while the same amount of EM SCEF provided a higher polyphenol content (20.84±0.04 mg·g<sup>-1</sup>) than the non-fermented extract. The extraction results for EM SCEF at 5, 10, 20, and 40 mg·mL<sup>-1</sup> were 25.82±0.04, 29.13±0.05, 42.07±0.05, and 59.22±0.09 mg·g<sup>-1</sup>, respectively.

In the case of the extract of ordinary SC, the corresponding results were 6.07±0.01, 11.87±0.01, 20.57±0.03, and 40.95±0.02 mg·g<sup>-1</sup>. So, the extract of EM SCEF had a higher content of polyphenol than found in the SCE group. As the concentration of the schisandra extract

**Table 3** Content of Polyphenols in SCE and SCEF

Content of SCE or SCEF (mg mL <sup>-1</sup> )	Polyphenols in SCE (mg g <sup>-1</sup> )	Polyphenols in SCEF (mg g <sup>-1</sup> )
1	1.53±0.02	20.84±0.04
5	6.07±0.016	25.82±0.04
10	11.87±0.01	29.13±0.05
20	20.57±0.03	42.07±0.05
40	40.95±0.02	59.22±0.09

increased, the polyphenol content in the extract also increased. When compared, the extract of the EM SCEF group showed a higher content than the SC group. This implies that SCE reacted with the EM active solution and led to the multiplication of polyphenols, which are a very useful physiological reactive substance for the human body. According to many reports by other researchers, there is a proportional relationship between antioxidant ability and content of flavonoids.<sup>28-30</sup> It was demonstrated that the flavonoid content depends on the concentrations of EM SCEF and SCE (Table 4). With low SCE and SCEF content, such as 1, 5, and 10 mg·mL<sup>-1</sup>, no flavonoid value was found. When the concentration in the EM SCEF group and the SCE group was 40 mg·mL<sup>-1</sup>, the flavonoid content was 2.79±0.02 and 1.59±0.08 mg·g<sup>-1</sup>, respectively. The results indicate that the EM SCEF has a high antioxidant ability.

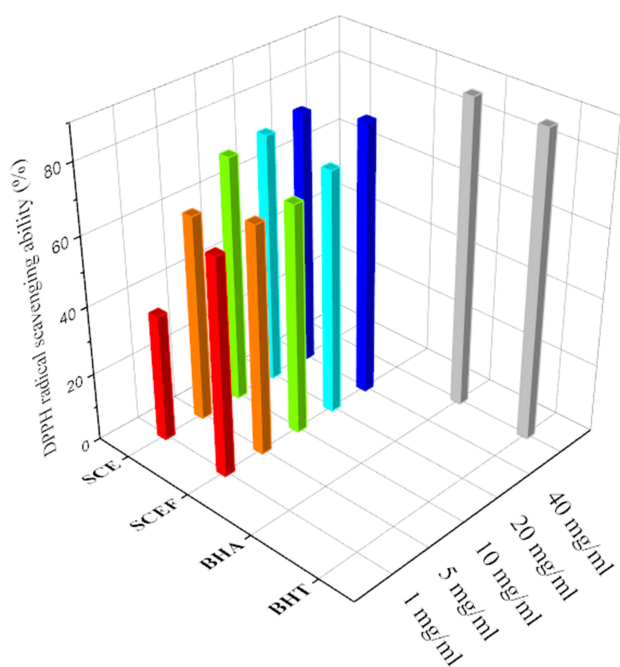
### Measurement of Free-Radical Scavenging

Free radicals in the body can promote biological aging, by reacting with lipids and proteins. To remove this phenomenon, many studies have investigated natural products.<sup>31</sup> The DPPH radical scavenging test method is used in many natural products for antioxidant measurements using the electron-donating ability of antioxidants.<sup>32-34</sup> The results of the antioxidant effects in the SCE and EM

**Table 4** Content of Flavonoids in SCE and SCEF

Content of SCE or SCEF (mg mL <sup>-1</sup> )	Flavonoids with SCE (mg g <sup>-1</sup> )	Flavonoids with SCEF (mg g <sup>-1</sup> )
1	0	0
5	0	0
10	0	0
20	0	1.46±0.04
40	1.59±0.08	2.79±0.02

SCEF groups are shown in Figure 1. In the case of the DPPH radical scavenging ability of SCE, as the concentration varied from 1.0, 10, to 40 mg·mL<sup>-1</sup>, the antioxidant ability increased from 37%, 72%, to 74%. In the EM SCEF group, showed 63%, 67%, and 79% antioxidant ability at the respective concentrations of 1.0, 10, and 40 mg·mL<sup>-1</sup>. In the EM SCEF group, the minor change in antioxidant ability with concentration seems to be due to the reaction between EM SCEF and microbes to produce antioxidant substances. The antioxidant ability of SC was compared to some well-known antioxidants, such as BHT (89%) and BHA (88%). It was found that the free-radical scavenging ability of SC was not much different from them. In addition, SCEF has higher radical scavenging ability than SCE, even at low concentrations. This means that as SC and EM active solutions reacted with each other, the microbes produced physiologically active



**Figure 1** DPPH radical scavenging ability of the SCE and SCEF.

materials which have antioxidant ability. Therefore, it is possible to produce material containing higher levels of antioxidants with lower amounts of EM SCEF than SCE. We concluded that this could solve a problem of dosage in manufacturing cosmetics, and simultaneously improve functional aspects of cosmetic products containing natural substances derived from plants.

### Measurement of Nitrite Scavenging Activity

Nitrite reacts with secondary amine (a chemical compound in which two hydrogen atoms of ammonia are substituted with the hydrocarbon functional group R), producing nitrosamine, a notorious carcinogen; in other words, nitrite acts as a precursor for nitrosamine. Therefore, the formation of nitrosamine can be effectively inhibited by removing nitrite.<sup>35</sup> If reactivity between the sample for analysis and nitrite is high, nitrite will be removed because it reacts in the ionized state, which leads to the inhibition of nitrosamine formation. This applies equally to other substances that exist in ion or electron forms, and the high reactivity of the sample is equivalent to or assessed as high activities of nitrite scavenging and antioxidation. The greater the amount of total phenol compounds in a sample, the more powerfully the reaction of nitrite scavenging occurs in the lower pH range, and, adversely, the scavenging effect decreases in upper pH range.<sup>36</sup> Table 5 shows that the nitrite scavenging activity of SCE was 15% at 1 mg·mL<sup>-1</sup>, 40% at 10 mg·mL<sup>-1</sup>, and 89% at 40 mg·mL<sup>-1</sup>. On the other hand, SCEF showed nitrite scavenging activity of 51% at 1 mg·mL<sup>-1</sup>, 69% at 10 mg·mL<sup>-1</sup>, and 98% at 40 mg·mL<sup>-1</sup>. From this test, it was observed that as the concentration of both groups increased, the scavenging activity also increased. In addition, SCEF was superior to SC in its nitrite scavenging activity, which is similar to other experimental results. At a concentration of 1.0 mg·mL<sup>-1</sup>, the difference in scavenging activity was 40 mg·mL<sup>-1</sup>, the largest among the different concentrations, and the gap narrowed as the concentration increased. From this comparative experiment, through the EM fermentation process, the nitrite scavenging effect of SC, which was assessed as being quite good, improved further. This phenomenon can be attributed to the fermentation process generating more biologically active substances, which, in turn, led to an increase in the inhibition of nitrosamine formation, as well as many phenols, as crude plant ingredients, also contributing to the effective nitrite scavenging reaction.

**Table 5** Nitrite Scavenging Ability of SCE and SCEF at pH 2.5

Content of SCE or SCEF (mg mL <sup>-1</sup> )	Nitrite Scavenging Activity with SCE (%)	Nitrite Scavenging Activity with SCEF (%)
1	15.53±0.32	51.85±0.23
5	30.36±0.15	64.68±0.15
10	40.00±0.15	69.01±2.96
20	61.80±0.16	84.33±0.23
40	89.39±0.08	98.75±0.26

## SODA Measurement

SOD is an enzymatic antioxidant which is able to detoxify and suppress the toxicity of O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub>, peroxide, OH radicals, etc.<sup>37,38</sup> SODA is shown in Table 6 for SCE (or SCEF) concentrations of 1.0, 10, and 40 mg·mL<sup>-1</sup>. The SCE group had SODA of 6%, 18%, and 41%, while the EM SCEF group had SODA of 28%, 32%, and 43% when the concentration increased from 1 to 40 mg·mL<sup>-1</sup>. To analyze the difference in activity of the two groups, the difference in SODA value was higher at a low concentration of SCE and SCEF (1.0 mg·mL<sup>-1</sup>) and smaller at a high concentration (40 mg·mL<sup>-1</sup>). In this test, both groups had an outstanding level of SODA.<sup>26,39</sup> Therefore, it can be judged that both SCE and SCEF have high, naturally originating, antioxidant ability.

## Measurement of Tyrosinase Inhibitory Activity

The mechanism of tyrosinase inhibitory activity is very important in the cosmetic industry and can be used as a measure of the skin whitening effect.<sup>40</sup> In the SCE group, tyrosinase inhibitory activity increased from 35% to 36%, 37%, 38%, and 39% as the concentration of the extract increased (Table 7). In the EM SCEF group, tyrosinase inhibitory activity increased from 38% to 39%, 40%, 41%, and 42% when the concentration increased. The EM SCEF had a more effective tyrosinase inhibitory activity than the

**Table 6** SOD-Like Activity of SCE and SCEF

Content of SCE or SCEF (mg mL <sup>-1</sup> )	SODA in SCE (%)	SODA in SCEF (%)
1	6.66±4.64	28.00±0.56
5	8.57±3.98	30.14±0.58
10	18.62±3.08	32.84±0.47
20	35.07±1.21	40.09±0.38
40	41.92±0.47	43.25±0.17

**Table 7** Inhibitory Effects of SCE and SCEF on In Vitro Melanin Synthesis by Tyrosinase

Content of SCE or SCEF (mg mL <sup>-1</sup> )	Inhibitory Effects with SCE (%)	Inhibitory Effects with SCEF (%)
1.0	35.76±0.23	38.88±0.48
5.0	36.29±0.32	39.72±0.25
10	37.14±0.05	40.35±0.36
20	38.46±0.01	41.33±0.54
40	39.31±0.34	42.54±0.05

normal extract, but there was not much difference. However, it is thought that both extracts have a skin whitening effect when they are used to make cosmetics.<sup>41</sup>

## Safety Evaluation

The formulas of cosmetics with different concentrations of SCE and EM SCEF, ie, 0.0, 1.0, 5.0, 10, 20, and 40 mg·mL<sup>-1</sup>, are shown in Figure 2. The manufactured cosmetics were formed in W/O dosage form by adding the aqueous phase to the oil phase. The pH value of the surface of human skin is generally between 4.5 and 6.5, which is either slightly acidic or neutral.<sup>42</sup> If the pH becomes alkaline, the skin's resistance will be weakened, leading to a propagation of germs and eventually to skin diseases. Therefore, it is highly recommended to use neutral or slightly acidic cosmetic products. The change in pH value with storage time is shown in Figure 3. Using the cream without SCE, the pH was slightly increased to 6.23 after 60 days compared to its initial value of 6.25. The cream products with SCE concentrations of 1.0, 5.0, 10, 20, and 40 mg·mL<sup>-1</sup> had initial pH values of 5.53, 3.87, 3.43, 3.15, and 3.03, respectively. These pH values did not change after 60 days. The EM SCEF-based creams with EM SCEF concentrations of 1.0, 5.0, 10, 20, and 40 mg·mL<sup>-1</sup> had initial pH values of 4.12, 3.46, 3.37, 3.15, and 2.98, respectively. Similar pH values were observed after 60 days. These results mean that there was no significant difference in pH change in either group, and when the concentration of the extract increased, the pH value decreased. These results imply that there were no safety problems in using these cosmetic products.

## Effect of Temperature on Cosmetic Stability

The evaluation of the effect of temperature on the stability of cosmetic products helps us to understand the chemical and physical changes of cosmetic products. It was found that several phenomena occurred, such as acidification,

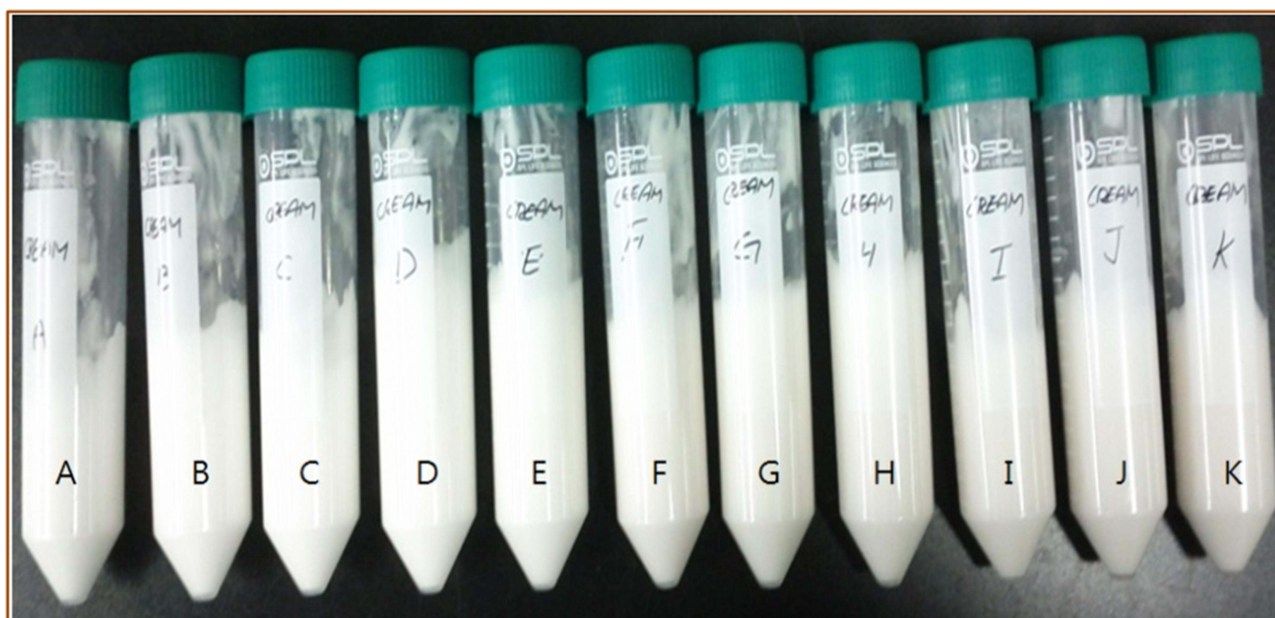


Figure 2 Photograph of the W/O emulsion containing SCE and SCEF.

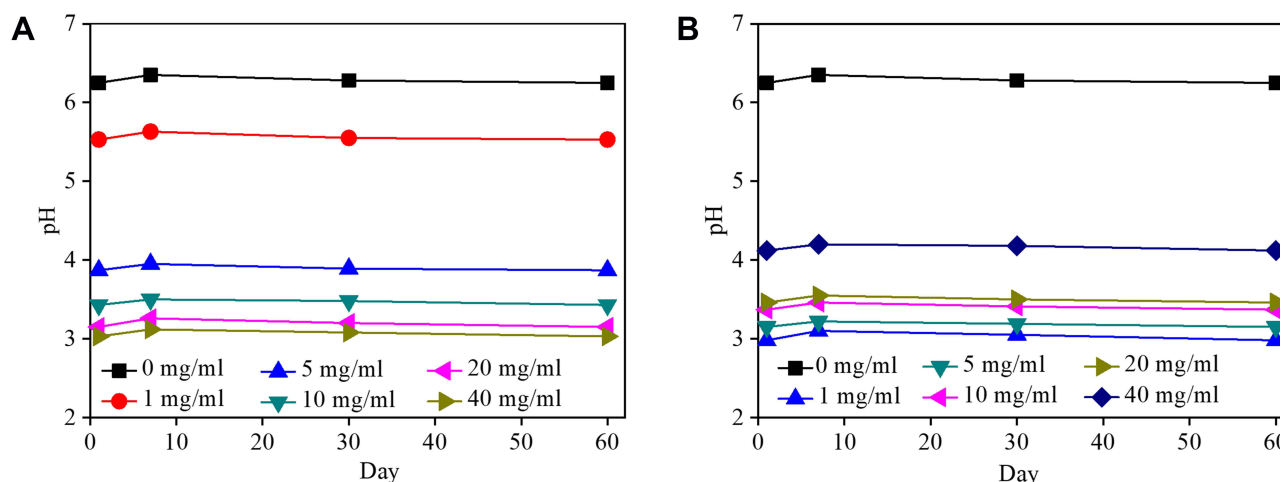


Figure 3 Change in pH values of the emulsion containing (A) SCE and (B) SCEF.

discoloration, evaporation, flotation, precipitation, turbidity, and separation at different temperatures in the cosmetic products after 60 days (Table 8).

### Conclusion

In this study, we successfully synthesized both SCE and SCEF, which provide a source of biologically active substances with potential application in the design of innovative natural cosmetics with a broad spectrum of activity. In case of the antioxidant activity test of SCE, DPPH, radical

scavenging, nitrite scavenging, and SODA measurement of SC by EM fermentation were the highest among the tested groups. The SCE and SCEF showed little differences in their skin whitening effect. The measurement stability and the safety evaluation showed that the obtained substances contain effective chemical components that have antioxidant activity, suppressing skin aging and having a whitening effect in a weak acid range consistent with a pH of 6.25–2.98; therefore, these substances can be a nominated as a material for future cosmetic application.



**Table 8** Stability Test of the Emulsions Containing SCE and SCEF at Different Temperatures

Temperature Day	0°C										
	A	B	C	D	E	F	G	H	I	J	K
1	O	O	O	O	O	O	O	O	O	O	O
2	O	O	O	O	O	O	O	O	O	O	O
3	O	O	O	O	O	O	O	O	O	O	O
4	O	O	O	O	O	O	O	O	O	O	O
5	O	O	O	O	O	O	O	O	O	O	O
Temperature Day	25°C										
	A	B	C	D	E	F	G	H	I	J	K
1	O	O	O	O	O	O	O	O	O	O	O
2	O	O	O	O	O	O	O	O	O	O	O
3	O	O	O	O	O	O	O	O	O	O	O
4	O	O	O	O	O	O	O	O	O	O	O
5	O	O	O	O	O	O	O	O	O	O	O
Temperature Day	40°C										
	A	B	C	D	E	F	G	H	I	J	K
1	O	O	O	O	O	O	O	O	O	O	O
2	O	O	O	O	O	O	O	O	O	O	O
3	O	O	O	O	O	O	O	O	O	O	O
4	O	O	O	O	O	O	O	O	O	O	O
5	O	O	O	O	O	O	O	O	O	O	O

Note: O, stable; X, unstable.

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## Disclosure

The authors report no conflicts of interest in this work.

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