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Keywords: wheat germ, hydrothermal extract, skin whitening, anti-wrinkle, tyrosinase, collagenase, trans-2-nonenal.

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ABSTRACT

Herein, we investigated the active ingredients in wheat germ as natural materials for functional cosmetics. Wheat germ hydrothermal extracts were obtained with 20 %, 50 %, and 80 % ethanol and used for GC-MS active ingredient analysis and antioxidant efficacy, whitening, wrinkle improvement, and trans-2-nonenal reduction rate analyses. Chromaticity analysis showed a rapid decrease in yellowness (b), which was believed to be attributed to the increased level of active substances having double bonds with increased ethanol content. GC-MS analysis identified high levels of hexadecanoic acid, 9, 12-octadecadienoic acid, and conjugated linoleic acid with increasing ethanol content. Also, low levels of linoleic acid ethyl ester and linoelaidic acid were detected. In contrast, as the ethanol content decreased, the active ingredients of cyclotrisiloxane were seen.*

Antioxidant efficacy analysis showed an IC_{50} value of 2.16 for ascorbic acid, which was the control, and 3.37, 3.39, and 2.76 for wheat germ extract with 20 %, 50 %, and 80 % ethanol, respectively.

All extracts displayed concentration-dependent antioxidant effects, although the effects were lower than that of ascorbic acid. Tyrosinase inhibition activity analysis showed that the extract with 80 % ethanol had the highest inhibition rate.

In collagenase inhibition activity analysis, all extracts had a similar level of inhibitory activity compared with ascorbic acid. These effects are thought to be attributed to the active ingredients of cyclotrisiloxane detected in the extract.

Additionally, trans-2-noneal removal analysis using GC-MS showed a high removal rate of 96.3 % in a wheat germ extract with 80 % ethanol.

Keywords: wheat germ, hydrothermal extract, skin whitening, anti-wrinkle, tyrosinase, collagenase, trans-2-nonenal.

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I. INTRODUCTION

Recently, there has been increasing interest in natural ingredients for food products and cosmetics, following the growing idea of naturalism for improved living standards and health. As demand for functional cosmetics is increasing, studies are actively investigating functional ingredients that can minimize the side effects of chemical ingredients on the skin, and natural extracts are being tested for their functional effects[1,2].

During aging, the skin undergoes various changes, including pigmentation and wrinkle formation. It produces melanin pigments to protect the internal and skin cells from ultraviolet (UV) rays.

However, excessive melanin production due to continuous UV exposure causes pigmentation, such as freckles and blemishes, accelerating skin aging. Melanin is first produced as tyrosine in melanocytes, which is oxidized by tyrosinase to become 3, 4-dihydroxy phenylalanine (DOPA).

DOPA is then converted to DOPA quinone, which is converted to melanin after various oxidative polymerization reactions[3]. Skin aging can be

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divided into intrinsic aging, caused by physiological aging, and extrinsic aging, caused by continuous UV exposure. Photoaging caused by UV exposure is considered the primary cause of skin aging [4]. Following skin aging, the production of structural proteins of the skin, such as collagen and elastin, is reduced, and collagenase biosynthesis is increased. This leads to greater expression of matrix metalloproteinases (MMPs), inducing matrix protein degradation in the dermis that deteriorates skin elasticity and results in skin sagging and wrinkles [5]. Additionally, impaired metabolism, along with skin aging, accumulates waste in the skin layers. As monounsaturated fatty acids in the skin surface lipids are decomposed, substances including nonenal aldehyde with a peculiar smell that are often observed in the elderly are generated [6,7]. 2-Nonenal is a long-chain aliphatic aldehyde containing nine carbons and unsaturated bonds. It is the primary cause of age-related, unpleasant, greasy, and sweaty odors and is found from age 40 onwards [8,9]. With aging, elderlies in their 60s and 70s have a slower, impaired olfactory function and fail to be aware of their scent. In particular, elderlies in their 60s and 70s have reduced absolute and relative sensitivity to smells, approximately 100 times lower than people in their 20s and 30s. [10].

According to the South Korea Ministry of Food and Drug Safety, products for skin whitening and wrinkle improvement are considered functional cosmetics. There are nine whitening materials registered with the Ministry of Food and Drug Safety, including arbutin, niacinamide, ascorbyl glucoside, mulberry extract, liquorice extract, and ethyl ascorbyl ether, and four types of raw materials, including adenosine, retinol, and polyethoxylated-retinide, which are known for wrinkle improvement effects [11]. However, there is still a lack of functional raw materials. Therefore, further studies must investigate functional materials for natural cosmetics that contain active ingredients for the skin while reducing the side effects caused by synthetic raw materials [12].

Additionally, studies on natural extracts are expected to induce technological and industrial growth. As there are sufficient demands for industrial development and consumer preference, the natural extracts must have competitive efficacy, safety, stability, and cost [13].

Wheat germ is produced as a by-product during the milling process of wheat. Pure wheat germ can be isolated for production due to its low cost and adequate demand and supply. Previous studies on germs of various grains, other than wheat, showed that polyphenols in barley germ have antioxidant effects [14,15]. Similarly, rice germs increased the protein content and enhanced the physiological activity through microbial fermentation [16]. Rice bran, a by-product of rice, lowered blood cholesterol level [17], and arabinoxylan extracted from rice bran inhibited cancer cell toxicity of natural killer (NK) cells [18]. Germs of Keunnunjami, a new rice variety, also have antioxidant effects [19,20].

Studies on wheat germ reported its anti- bacterial [21], anti-inflammatory [22], antioxidant [23], and immune-enhancing [24] effects. The wheat germ fat contains a high level of tocopherol, which makes it a valuable, healthy, functional food [25].

However, most studies on wheat germ focused on food materials, and there is a lack of studies on wheat germ as a cosmetic raw material. Therefore, this study aimed to analyze the antioxidant, whitening, wrinkle improvement, and trans-2-nonenal reduction effects of wheat germ hydrothermal extract through chromaticity analysis and GC-MS active ingredient analysis to confirm its functionality as a cosmetic material.

II. EXPERIMENT

2.1 Material

The wheat germ used in this study was provided by DAEHAN FLOUR MILLS CO., LTD., in November 2021.

2.2 Wheat Germ Extraction Condition

Wheat germ powder was used for the hydrothermal extraction of wheat germ. Water and ethanol were used as the solvent, and hydrothermal extraction was conducted with 20 %, 50 %, and 80 % ethanol. The total volume of the solvent was 300 ml, which was mixed with 30 g of wheat germ. A high-pressure hydrothermal extractor (KSP-240L, KYUNGSEO E&P, Inch eon, Korea) was used at 80 °C and 0.06 MPa for 3 h extraction. The extracted solution was decompression-filtered (DOA-P704-AC, G AST Manufacturing Inc., U.S.A) using a 90 mm filter (20 HM Hyundai Micro, Korea) and decompression-concentrated (EYELA N-1300, SHANGHAI EYE LA CO., China) for storage at 4 ℃.

The weight of the extracted sample after filtration under reduced pressure containing ethanol and the weight after concentration by completely evaporating the ethanol was used to calculate the extract yield (%) as follows.

$$Extract\ yield\ (\%) = \frac{Weight\ after\ concentration}{Weight\ after\ decompression} \times 100$$

2.3 Chromaticity Analysis of Wheat Germ Extract

The wheat germ extracts with varying ethanol contents analyses using a colorimeter (CR-400, Konica Minolta, Tokyo, Japan) was repeated three times, and the mean value was calculated. The value was expressed as L*(brightness), a*(redness), and b*(yellowness).

2.4 GC-MS Analysis of Wheat Germ Extract

GC-MS (Gas chromatography-mass spectrometry) was performed to analyze the active ingredients of wheat germ extract. HP-5ms (30 m x 250 µm x 0.25 µm) column (Agilent 19091S-433UI: 1456957H, Agilent technology, U.S.A) HP-5ms (30 m x 250 µm x 0.25 µm) was used for GC analysis. The carrier gas was helium, and the pressure and flow rate were set to 7.0699 psi and 1 mL/min, respectively. The temperature range of the column was −60–325 °C, and the oven temperature was 40–300 °C. GC-MS was conducted for a total of 45 min.

2.5 DPPH Scavenging Activity Analysis of Wheat Germ Extract

DPPH scavenging activity was measured using 95 % powder DPPH standard sample 2,2-Diphenyl-1-picrylhydrazyl (Alfa Aesar, Massachusetts, USA) (free radical) to assess the antioxidant effects of wheat germ extract. After diluting 5 g of all extracts with an ethanol content of 20 %, 50 %, and 80 % in 100 ml ethanol, the solution was re-diluted in ethanol at varying concentrations of 5 %, 10 %, 15%, 20 %, and 30%.

Then, 1.5 mM DPPH solution dissolved in methanol, wheat germ extract diluted at different concentrations, and ethanol were mixed in a ratio of 6 (600 µl): 3 (300 µl): 1 (100 µl) and reacted for 20 min in the absence of light. Absorbance was measured at 517 nm with a UV/Vis Spectrophotometer (KLAB, Deajeon, Korea).

Ascorbic acid was used as the control substance, and 0.1 mg/ml of ascorbic acid was mixed in an identical ethanol ratio to the wheat germ extract for identical testing conditions. The inhibition rate of DPPH Radical scavenging activity, indicated as IC50, was calculated as follows.

$$Inhibition\ rate\ (\%) = \frac{Abs\ (control) - Abs\ (sample)}{Abs(control)} \quad bs_{sample} = Abs_{test} - Abs_{color}$$

2.6 Analysis of Tyrosinase Inhibition Rate by Wheat Germ Extract

The tyrosinase inhibition activity on the whitening effect of wheat germ extract was evaluated. A substrate solution (0.2 ml), 0.02 g of 10 mM L-DOPA (3,4-Dihydroxy-L-phenylalanine, Sigma Aldrich, St. Louis, USA), 0.4 ml of the buffer, 0.2 ml of wheat germ extract, and 125 U/ml 0.2 ml of enzyme mushroom tyrosinase (T3824-25KU, Sigma Aldrich, St. Louis, USA) were added to 10 ml of 67 mM sodium phosphate buffer (pH 6.8).

The mix was stirred and reacted at 25 ℃ for 30 min. After centrifugation, the supernatant was transferred to a quartz cuvette (Quartz Cell, KMS, Korea). The absorbance of DOPA Chrome produced in the reaction solution was measured at 475 nm using a UV/Vis Spectrophotometer.

Kojic acid was used as a positive control. A total of 0.002 g of Kojic acid was added to 10 ml of DW, and the same experiment was performed. The control group without wheat germ extract and the experimental group with wheat germ extract were compared to calculate the tyrosinase inhibition rate.

$$Inhibtion\ rate\ (\%) = \frac{Extract\ sample\ O.D}{Control\ group\ O.D} \times 100$$

2.7 Analysis of Collagenase Inhibition Rate by Wheat Germ Extract

Collagenase inhibition activity dependence on wheat germ extract was analyzed as follows. A mixture of 0.1 M tris and 4 mM CaCl2 was mixed with buffer (pH 7.5) with 1 M HCl, 0.25 ml of 1.2 mg/ml 4-phenylazo benzyloxycarbonyl Pro-Leu-Gly-Pro-D-Arg (Sigma Aldrich, St. Louis, MO, USA) substrate solution, 0.1 ml of wheat germ extract, and 0.15 ml of buffer with 0.4 mg/ml of collagenase (CD130-100MG, Sigma Aldrich, St. Louis, U.S.A). The final mixture was reacted at 37 ℃ for 30 min and mixed with 0.5 ml of 20 % citric acid diluted with DW and 2.4 ml of ethyl acetate to terminate the reaction. After stirring and centrifugation at 120 RPM for 10 min (HA-12, Hanil Industrial, Incheon, Korea), the supernatant was transferred to a quartz cuvette, and the absorbance was measured at 320 nm using a UV/Vis Spectrophotometer. Ascorbic acid at a concentration of 0.1 g/ml was used as a positive control, and an identical experiment was performed.

The control group without wheat germ extract and the experimental group with wheat germ extract were used to calculate the collagenase inhibition rate with the following formula.

$$Inhibition\ rate\ (\%) = \frac{Extract\ sample\ O.D}{Control\ group\ O.D} \times 100$$

2.8. Analysis of trans-2-nonenal reaction rate by wheat germ extract using GC-MS

A reference solution was prepared using 100 ml of ethanol and 9 μl of Trans-2-Nonenal (TOKYO CHEMICAL INDUSTRY, Tokyo, Japan). Then, 1000 ul of the reference solution was mixed with 6, 6, and 4 ul of wheat germ extract with 20 %, 50 %, and 80 % ethanol, respectively. The mix was centrifuged and dispersed at 500 RPM for 30 min.

Subsequently, 150 ul of Fehling solutions A and B (Daejeong Chemical Co., Ltd., Siheung, Korea) with aldehyde group reducibility were added and incubated in an oven at 60 ℃ for 15 min. The mix was centrifuged, and the supernatant was used for GC analysis (Agilent 123-7063). The oven temperature was maintained at 50 ℃ for 3 min and increased by 5 ℃/min to a final temperature of 240 ℃, which was maintained for 2 min for analysis.

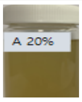
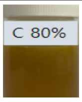
III. RESULTS AND DISCUSSION

The results of this study on wheat germ extract are displayed below. The notations for wheat germ extract with 20 %, 50 %, and 80 % ethanol are A, B, and C, respectively.

3.1 Wheat Germ Extract Yield

As shown in Table 1, the yield of wheat germ extracts according to the extraction conditions tended to decrease rapidly as the ethanol content increased. As ethanol's boiling point is 78 ℃, the set temperature of 80 ℃ during hydrothermal extraction may have been attributed to a decreased yield of wheat germ extract with increasing ethanol content.

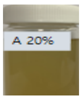
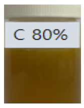
Table 1: Yield table for wheat germ extracts

No.	DW:EtOH	Extract	After decompression (g)	After concentration (g)	Yield (%)
A	80 : 20		110	79.4	72.2
B	50 : 50		211	135	64.0
C	20 : 80		172	38.8	22.6

3.2 Colorimetric Measurement of Wheat Germ Extract

Table 2 shows that increasing the ethanol content decreased brightness (L*) while increasing redness (a*) and yellowness (b*). In particular, yellowness tended to grow rapidly, which is thought to be related to the gradual increase in active substances with double bonds, following increased ethanol content.

Table 2: Colorimetric Results for Wheat Germ Extract

No.	A	B	C
			
L* (brightness)	23.6	20.6	15.1
a* (redness)	0.95	0.70	0.58
b* (yellowness)	-0.38	5.55	10.0

3.3 Analysis Result of Wheat Germ Extract Active Ingredients

GC-MS analysis identified various active ingredients as functional cosmetic materials in wheat germ extract. A comparison of wheat germ extract with ethanol content showed low levels of active ingredients in extract A in contrast to high levels of active ingredients detected in extracts B and C. The results are shown in Figures 1–3.

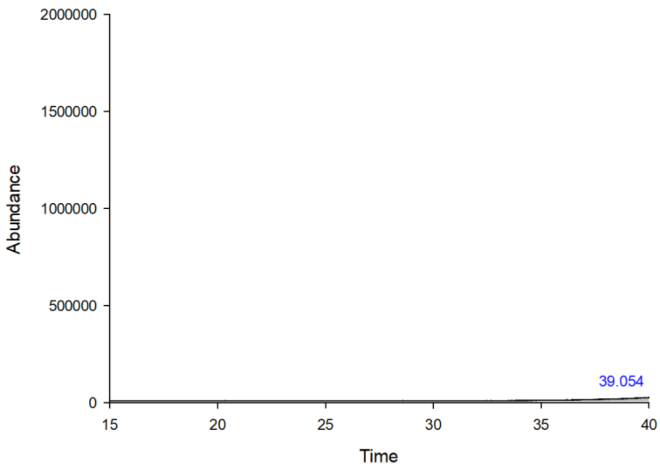


Figure 1: GC-MS Chromatogram of 20% Ethanol Extracts of Wheat Germ

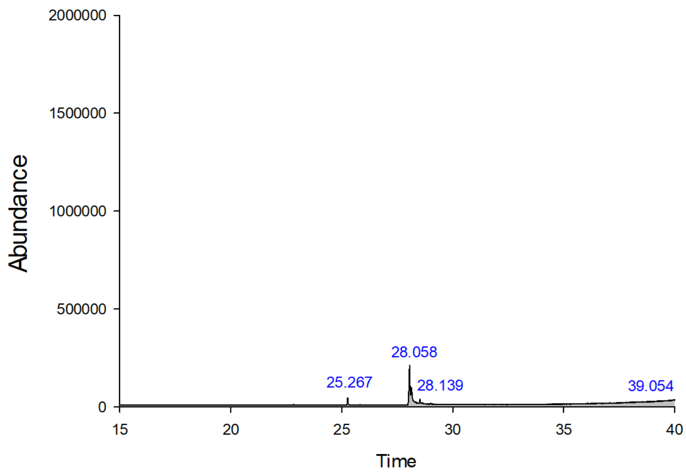


Figure 2: GC-MS Chromatogram of 50% Ethanol Extracts of Wheat Germ

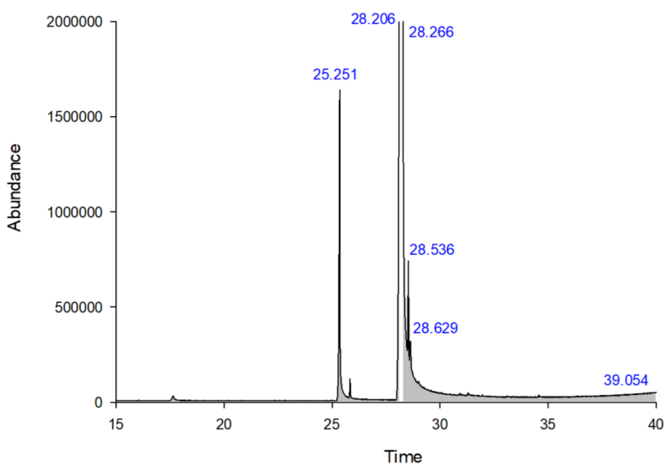


Figure 3: GC-MS Chromatogram of 80% Ethanol Extracts of Wheat Germ

As shown in Tables 3–5, extracts with higher ethanol content were associated with more active ingredients. In particular, in extract A, cyclotrisiloxane, an antibacterial and antioxidant active ingredient, was detected. In extract B, hexadecanoic acid, conjugated linoleic acid, and 9, 12-octadecadienoic acid with antioxidant effects were detected. In extract C, in addition to hexadecanoic acid, conjugated linoleic acid, and 9, 12-octadecadienoic acid, linoleic acid ethyl ester, and linoelaidic acid were also identified.

Table 3: GC-MS chromatogram profile of 20% ethanol of wheat germ

Retention time (RT)	Name of the compound	Quality	Area peak (%)
39.054	Cyclotrisiloxane	47	17.0

Table 4: GC-MS Chromatogram Profile of 50% Ethanol of Wheat Germ

Retention time (RT)	Name of the compound	Quality	Area peak (%)
25.267	Hexadecanoic acid	99	7.84
28.058	9,12-Octadecadienoic acid	99	53.3
28.139	Conjugated linoleic acid	95	38.8
39.054	Cyclotrisiloxane	47	18.0

Table 5: GC-MS Chromatogram Profile of 80% Ethanol of Wheat Germ

Retention time (RT)	Name of the compound	Quality	Area peak (%)
25.251	Hexadecanoic acid	99	8.33
28.206	9,12-Octadecadienoic acid	99	57.7
28.266	Conjugated linoleic acid	99	27.5
28.536	Linoleic acid ethyl ester	99	3.34
28.629	Linoelaidic acid	97	2.71
39.054	Cyclotrisiloxane	47	19.0

3.4 Analysis Result of Wheat Germ Extract Antioxidant Efficacy

Antioxidation analysis using DPPH was based on the property that 1.1-diphenyl-2-picrylhydrazyl (DPPH) is a relatively stable free radical with an absorption band at 517 nm. DPPH, in purple, is reduced and discolored to yellow when it encounters electrons or hydrogen radicals from substances with antioxidant effects, and the absorbance of the reduced value was measured26]. DPPH radical scavenging activity of wheat germ extract was assessed. Ascorbic acid in the control group had an IC50 of 2.16. In extracts A, B, and C, IC50 was 3.37, 3.39, and 2.76, respectively. Thus, all samples showed antioxidant effects in a concentration-dependent manner (Figure 4), although the effects were lower than that of control ascorbic acid. In particular, extract C showed more excellent antioxidant effects, which may be attributed to the high levels of antioxidant active ingredients in extract C compared with extracts A and B.

Table 6: Half maximal inhibitory concentration (IC50)

No.	IC_{50}
Ascorbic acid	2.16
A	3.37
B	3.39
C	2.76

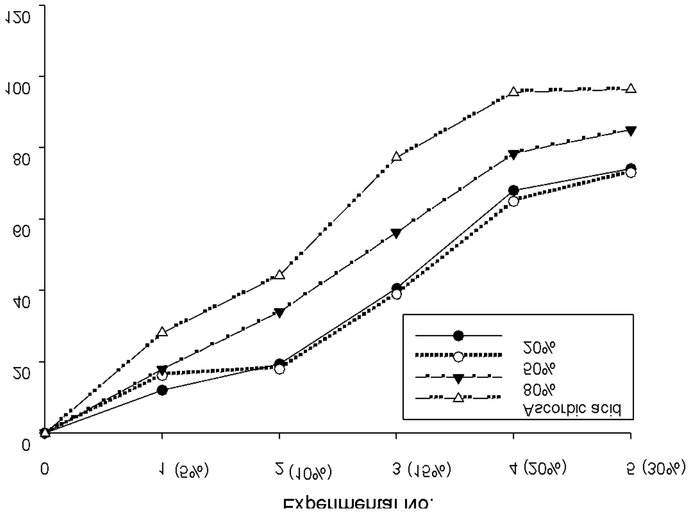


Figure 4: Antioxidant ability according to extraction conditions

3.5 Analysis Result of Tyrosinase Inhibitory Activity by Wheat Germ Extract

Figure 5 shows tyrosinase inhibition activity for each wheat germ extract. As the ethanol content increased, the tyrosinase inhibition rate increased. In particular, extract C with 80 % ethanol showed an inhibition rate greater than 80 %, suggesting more excellent whitening effects compared with that of the control Kojic acid. The effects are thought to be mediated by the 9, 12-octadecadienoic acid, linoleic acid ethyl ester, linoelaidic acid, and conjugated linoleic acid.

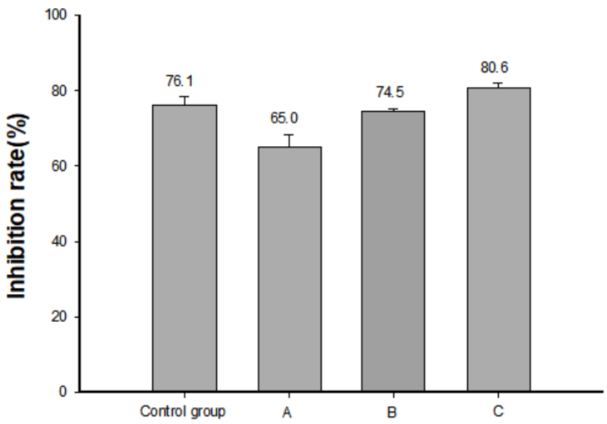


Figure 5: Effect of wheat germ extracts on tyrosinase inhibitory activity

3.6 Analysis Result of Collagenase Inhibitory Activity by Wheat Germ Extract

Collagen does not react to proteolytic enzymes; however, studies reported that collagen is degraded by collagenase. Reduced collagenase activity is essential in lowering skin elasticity, therefore suppressing wrinkle formation. Wrinkle improvement by wheat germ extract is shown in Figure 6. All extracts showed a similar collagenase inhibition rate as ascorbic acid. It is thought that the active ingredient, cyclotrisioloxane, identified in GC-MS component analysis, mediates these effects.

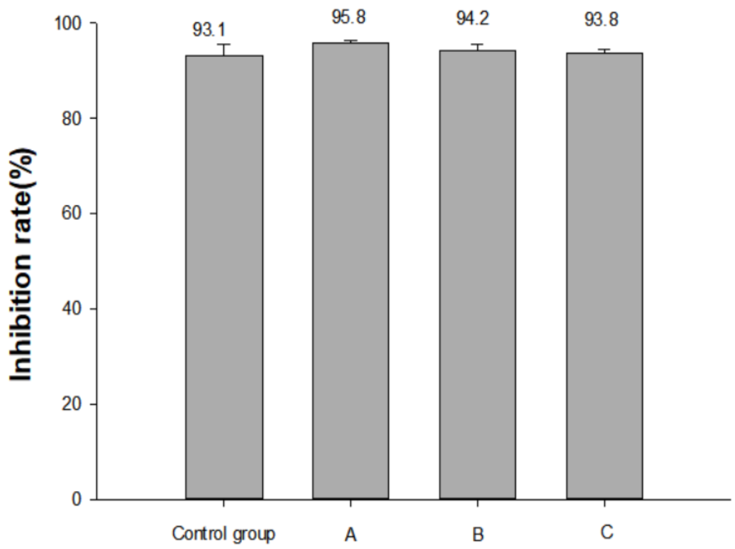


Figure 6: Effect of Wheat Germ Extracts on Collagenase Inhibitory Activity

3.7 Analysis Result of Trans-2-Nonenal Removal by Wheat Germ Extract Using GC-MS

Table 7 and Figure 7 show the overall removal rate of trans-2-nonenal analyzed using GC-MS. The removal rate was 83.5 % in extract A, 94.9 % in extract B, and 96.3 % in extract C, which indicated high trans-2-nonenal removal effects by wheat germ extract. This is thought to be attributed to the high levels of active ingredients, 9, 12-octadecadienoic acid, and conjugated linoleic acid, identified in the extract.

Table 7: GC-MS Results for the Evaluation of Trans-2-Nonenal Removal Efficacy of Extracts by Extraction Conditions

	Response	Final conc.	Removal rate(%)
Control	12253		
A	2014	<0.01	83.5
B	613	<0.01	94.9
C	452	<0.01	96.3

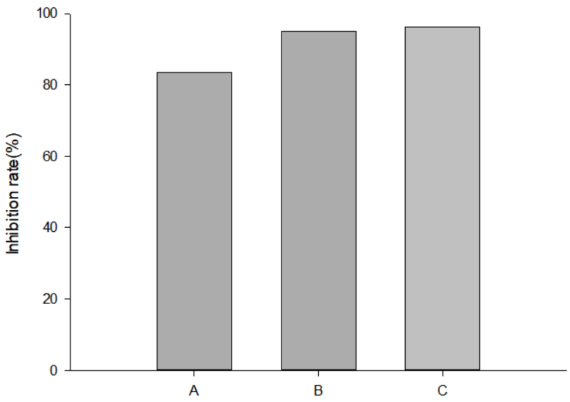


Figure 7: Comparison of GC-MS Results for the Evaluation of Trans-2-Nonenal Removal Efficacy of Extracts According to Extraction Conditions

IV. CONCLUSION

The results of wheat germ hydrothermal extract by ethanol content were as follows:

1. Chromaticity analysis showed decreased brightness (L*) and increased redness (a*) and yellowness (b*). In particular, yellowness tended to increase rapidly, which is thought to be related to the increasing level of effective active ingredients with double bonds as the ethanol level increased.
2. Qualitative analysis of GC-MS indicator components of wheat germ extract showed that as ethanol content increased, the active ingredient increased and was detected at high levels. In extract A, only a low level of the active ingredient of cyclotrisiloxane was detected. In contrast, in extracts B and C, high levels of active ingredients, such as hexadecanoic acid, conjugated linoleic acid, and 9,12-Octadecadienoic acid, were detected.
3. DPPH radical scavenging activity of wheat germ extracts were as follows: IC50 of ascorbic acid, extract A, extract B, and extract C were 2.16, 3.37, 3.39, and 2.76, respectively. All extract samples had lower DPPH radical scavenging activity than ascorbic acid; however, the extract showed concentration-dependent antioxidant effects.
4. Tyrosinase inhibition activity of wheat germ extract increased with ethanol content. In particular, extract C showed a high tyrosinase inhibition rate more excellent than 80 %, suggesting it had greater whitening effects than control Kojic acid. The effects are thought to be mediated by the 9, 12-octadecadienoic acid, linoleic acid ethyl ester, linoelaidic acid, and conjugated linoleic acid.
5. In all extracts, collagenase inhibition activity was similar compared with that in ascorbic acid. This may be attributed to the active ingredient, Cyclotrisiloxane, identified in the extract.
6. Analysis of trans-2-nonenal removal rate by wheat germ extract using GC-MS showed a rate of 83.5 %, 94.9 %, and 96.3 % in extracts A, B, and C, respectively, suggesting a high trans-2-noneal removal rate. The effects are thought to be mediated by the 9, 12-octadecadienoic acid, linoleic acid ethyl ester, linoelaidic acid, and conjugated linoleic acid.

Herein, our findings demonstrated that wheat germ hydrothermal extract contains natural active ingredients for functional skin cosmetics. Wheat germ extract effectively removed trans-2-nonenal, the critical cause of unpleasant odor in the elderly, whilst whitening the skin and improving wrinkles. Therefore, wheat germ extract can be potentially used as a natural material for functional cosmetics.

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