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Research Article
Exploring Physiologically Active Compound Variations in *Angelica dahurica* and *Elsholtzia splendens* through Fermentation with Diverse Strains and Conditions

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Abstract

Background and Objective: In recent times, there has been a growing demand for the development and utilization of natural compounds, as opposed to synthetic substances. This study investigates the changes in the content of physiologically active compounds, namely apigenin, luteolin, nodakenin and scopoletin, in *Angelica dahurica* and *Elsholtzia splendens* through fermentation with various strains and conditions. **Materials and Methods:** *Angelica dahurica* and *Elsholtzia splendens* were extracted by using 70% ethanol and conducted for fermentation. Fermentation was carried out using strains of *Lactobacillus casei*, *Bacillus subtilis*, *Saccharomyces cerevisiae* and *Aspergillus oryzae*, employing both liquid and solid-state fermentation techniques with diverse temperature settings. High-Performance Liquid Chromatography (HPLC) was employed for the analysis of apigenin, luteolin, nodakenin and scopoletin content. **Results:** This research confirms significant variations in the levels of physiologically active compounds based on fermentation strains, states and temperatures. For *Angelica dahurica*, there is a notable trend of increased content of all compounds when *Saccharomyces cerevisiae* is utilized in liquid fermentation and a similar trend is observed primarily when either *Saccharomyces cerevisiae* or *Aspergillus oryzae* is used in solid-state fermentation. The result of *Elsholtzia splendens* is similar to *Angelica dahurica*. **Conclusion:** The outcomes of this study provide fundamental insights into fermentation conditions that enhance the levels of physiologically active compounds in *Angelica dahurica* and *Elsholtzia splendens*.

Key words: *Angelica dahurica*, *Elsholtzia splendens*, fermentation, physiologically active compound

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In recent years, research on the development and utilization of substances with minimal side effects derived from natural products rather than synthetic compounds, has gained significant attention. Additionally, research focused on enhancing the efficacy by bio-transforming active ingredients through the fermentation of natural products is a growing trend[1-3].

Fermentation is a metabolic transformation process of natural substances by intestinal microorganisms, which is known to convert plant glycoside compounds into their active form, referred to as aglycones. This conversion leads to the biological transformation of active compounds and aims to enhance the absorption rate of these compounds within the body while regulating their pharmacological activities[4-6].

Angelica dahurica (*A. dahurica*), belonging to the Apiaceae family, is reported to possess various pharmacological effects such as anti-oxidant, anti-microbial, anti-inflammatory and anti-cancer properties, attributed to its roots[7-13]. *Elsholtzia splendens* (*E. splendens*), a member of the Labiatae family, is known for its diverse therapeutic effects including anti-oxidant, anti-inflammatory, anti-cancer and improvement of premenstrual syndrome[14-18]. The pharmacological activities of *A. dahurica* and *E. splendens* are attributed to various physiologically active compounds present in them.

Effective compounds in *A. dahurica* and *E. splendens* include apigenin, luteolin, nodakenin, scopoletin, among others. Apigenin, a type of flavonoid, has been extensively studied for its outstanding anti-oxidant, anti-aging, anti-inflammatory and anti-cancer effects[19-21]. Luteolin, another flavonoid, demonstrates anti-oxidant, anti-inflammatory and anti-cancer actions, with various physiological benefits such as protecting the liver from toxic substances[22-24]. Nodakenin, a coumarin derivative, is widely reported for its anti-oxidant and anti-microbial properties and is suggested to contribute to uterine health[25-28]. Scopoletin, a coumarin derivative, is particularly known for its excellent anti-inflammatory effects[29-33].

Research on the active compounds of natural products has been ongoing for a long time. However, despite the fact that optimal extraction conditions for these active compounds can vary depending on the extraction method, fermentation microorganisms and other variables, research on the transformation of compounds through the fermentation of natural products is still relatively limited. Therefore, studies aimed at determining the optimal extraction conditions for active compounds are essential. In this study, by comparing the changes in apigenin, luteolin, nodakenin and scopoletin in the fermentation extracts and organic solvent extracts of *A. dahurica* and *E. splendens*, we aim to provide fundamental data for the development of plant-derived drugs.

MATERIALS AND METHODS

Study area: The study was carried out at Sooy-K Bio Lab and University of Eulji in 2023.

Extraction of *A. dahurica* and *E. splendens*: The *A. dahurica* and *E. splendens* used in the experiments were purchased from Herbal Medicine Market (Jecheon, Korea). They were cleaned, separated from contaminants, dried and then ground using a grinder (HMFP-30000, Hanil, Korea). The resulting samples were stored in a cool, dry place for use in extraction and fermentation.

Extraction was carried out by adding 400 mL of 70% ethanol to 100 g of the ground *A. dahurica* and *E. splendens* and the mixture was allowed to extract at room temperature for 48 hrs. The extract was then filtered through filter paper (No. 2, Hyundai Micro, Korea) and concentrated at 45°C using a rotary vacuum evaporator (Rotavapor R-215, Buchi, Germany). The concentrated powder was stored at -50°C for later use. The yield of the concentrated powder was calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{Initial material weight (g)}}{\text{Dried powder weight (g)}} \times 100$$

Fermentation strains: The fermentation strains utilized in the experiment were organized in Table 1. *Lactobacillus casei* (*L. casei*), *Bacillus subtilis* (*B. subtilis*) and *Saccharomyces cerevisiae* (*S. cerevisiae*) were acquired from the Microbiology Laboratory at Suwon University for experimental purposes. *Aspergillus oryzae* (*A. oryzae*) was isolated from yeast and identified through 18S rRNA analysis at the Korean Culture Center of Microorganisms.

Subsequently, *L. casei* on MRS agar (Difco Co., USA), *B. subtilis* was cultured on Nutrient agar (Difco Co., USA), *S. cerevisiae* on Yeast Malt agar (Difco Co., USA) and *A. oryzae* on Potato Dextrose agar (Difco Co., USA) for the experiments.

Selection of optimal growth conditions for fermentation strains: The *L. casei* strain was activated by culturing on MRS agar plates at 37°C for 24 hrs. The *B. subtilis* strain was activated by culturing on Nutrient agar plates at 37°C for 24 hrs, while the *S. cerevisiae* strain was activated by culturing

on Yeast Malt agar plates at 30°C for 48 hrs. After activation, a small portion (1 loopful) of the activated strains was inoculated and cultured for 24 hrs. The culture medium was adjusted to pH 6.0, sterilized and then inoculated with 200 µL of the bacterial solution. The cultured medium was diluted 100 times and cultured for 1 or 2 days at 30 and 37°C. Cell density was determined using the spread plating method and the optimal growth conditions were identified by counting colonies using a colony counter (PROTOS3, Synbiosis, USA).

Fermentation conditions and preparation of fermentation extracts: Liquid fermentation was conducted by adding 6 g of *A. dahurica* and *E. splendens* to 100 mL of optimal growth medium for each strain and sterilizing at 121°C and 1.5 atmospheric pressure. The *L. casei, B. subtilis* and *S. cerevisiae* were activated according to their optimal growth conditions, inoculated into the sterilized mixture at 1% (v/v) and fermented for 2 days at 30°C. The liquid fermentation products of *A. dahurica* and *E. splendens* were dried for 2 days at 50°C and then extracted at room temperature for 48 hrs with 8 times the volume of 70% ethanol.

Solid-state fermentation involved mixing 100 g of *A. dahurica* and *E. splendens* with 50% moisture, sterilizing the fermentation raw material, incubating at room temperature, inoculating with 1 mL of fermentation strains evenly distributed and fermenting for 7 days. The *L. casei*, *B. subtilis* and *S. cerevisiae* were fermented at 30°C and 37°C, while *A. oryzae* was fermented at 25 and 30°C. After fermentation, the extracts were obtained by adding 4 times the volume of 70% ethanol and extracting at room temperature for 48 hrs. The liquid and solid-state fermentation extracts were filtered through filter paper concentrated using a rotary vacuum evaporator at a water bath temperature of 45°C and stored as concentrated powder at -50°C for experimental use.

HPLC analysis conditions: The content of apigenin, luteolin, nodakenin and scopoletin in *A. dahurica* and *E. splendens* extracts and fermentation extracts was analyzed using an Agilent 1100 DAD/UV HPLC system (Agilent, USA). The column used was Capcell Pak C18 UG120 5 µm, 4.6×250 mm (Shiseido, Japan), with a temperature set at 30°C. The mobile phase employed distilled water containing 0.1% acetic acid and acetonitrile containing 0.1% acetic acid, with a flow rate set at 1 mL/min. Samples of 10 µL each were injected and the analysis was carried out for 60 min. Detection wavelengths were set at 320 nm for apigenin, 336 nm for luteolin, 320 nm for nodakenin and 345 nm for scopoletin. Standard substances purchased from Avention (USA) were used to construct calibration curves for the quantification of apigenin, luteolin, nodakenin and scopoletin.

RESULTS AND DISCUSSION

Optimization of fermentation strain growth conditions: To facilitate efficient fermentation using *A. dahurica* and *E. splendens*, the growth conditions of the fermentation strains were systematically investigated for optimal performance and shown in Table 2. By varying the microbial cultivation conditions, the yield of each strain was examined under different circumstances. Conditions for the cultivation of starter strains and the fermentation of *A. dahurica* and *E. splendens* were meticulously selected based on these investigations. The *L. casei*, *B. subtilis* and *S. cerevisiae* exhibited a consistent trend of higher cell yields at 30°C compared to 37°C, for the same cultivation period. Furthermore, *L. casei* and *S. cerevisiae* demonstrated higher cell yields when cultured for 2 days compared to 1 day. Although *B. subtilis*, at 30°C, mirrored other strains by showing higher cell yields after 2 days of cultivation, a different trend emerged at 37°C, where a higher cell yield was observed after 1 day. Under the optimized conditions of 2 days cultivation at 30°C, *L. casei*, *B. subtilis* and *S. cerevisiae* exhibited elevated cell yields of 5.15×10^9, 1.82×10^9 and 1.78×10^8 CFU/mL, respectively, surpassing yields under other conditions. Based on these results, the optimal growth conditions for fermentation strains were selected to facilitate the subsequent fermentation process effectively.

Yield of *A. dahurica* and *E. splendens* fermentation extracts: Liquid and solid fermented extracts, obtained through fermentation with various strains, cultivation temperatures and durations, underwent extraction using 70% ethanol, followed by concentration and powdering. The resulting variations in yield, based on these fermentation conditions, were thoroughly investigated.

For *A. dahurica*, a trend of higher yields in liquid fermentation compared to solid-state fermentation was observed. Under solid-state fermentation conditions, fermented extract obtained from *S. cerevisiae* showed lower yield compared to other strain conditions, while *L. casei*, *B. subtilis* and *A. oryzae* fermented extracts all exhibited yields of 20% or more. Furthermore, all strain conditions showed higher yields at 37°C compared to 30°C. In liquid fermentation conditions, yields of *L. casei*, *B. subtilis* and *S. cerevisiae* fermented extracts were 43.55, 29.79 and 15.94%, respectively. Notably, extracts from *L. casei* and *B. subtilis* fermentations showed increased yields compared to the control group at 23.53% (Table 3).

In the case of *E. splendens*, under solid-state fermentation conditions, fermented extracts obtained from *S. cerevisiae* and *A. oryzae* showed lower yields compared to those

Table 1: Strains used for fermentation

Strains	Sources	Growth medium
Lactobacillus casei	Doenjang	Nutrient agar
Bacillus subtilis	Cheese	MRS agar
Saccharomyces cerevisiae	Grapes	Yeast Malt agar
Aspergillus oryzae	Fermented soybeans	Potato Dextrose agar

Table 2: Comparison of viable cell according to culture conditions

	Fermentation conditions		
Strains	Temperature (°C)	Time (days)	Viable cell (CFU/mL)
Lactobacillus. casei	30	1	3.50×10^{9}
	30	2	5.15×10^{9}
	37	1	2.93×10^{9}
	37	2	1.19×10^{9}
Bacillus subtilis	30	1	1.43×10^{8}
	30	2	1.82×10^{9}
	37	1	1.01×10^{8}
	37	2	6.50×10^{8}
Saccharomyces cerevisiae	30	1	1.62×10^{8}
	30	2	1.78×10^{8}
	37	1	9.10×10^{7}
	37	2	1.10×10^{8}

Table 3: Yields of 70% ethanol extract after fermentation of *Angelica dahurica* and *Elsholtzia splendens*

		Fermentation conditions		
State	Strains	Temperature (°C)	Time (days)	Extract yields (%, w/w)
A. dahurica				
Liquid	*L. casei*	30	1	43.55
Liquid	*B. subtilis*	30	1	29.79
Liquid	*S. cerevisiae*	30	1	15.94
Solid	*L. casei*	30	7	20.57
Solid	*L. casei*	37	7	22.52
Solid	*B. subtilis*	30	7	20.21
Solid	*B. subtilis*	37	7	22.80
Solid	*S. cerevisiae*	30	7	11.13
Solid	*S. cerevisiae*	37	7	12.51
Solid	*A. oryzae*	25	7	21.42
Solid	*A. oryzae*	30	7	21.98
Control group				23.53
E. splendens				
Liquid	*L. casei*	30	1	20.77
Liquid	*B. subtilis*	30	1	9.32
Liquid	*S. cerevisiae*	30	1	4.18
Solid	*L. casei*	30	7	8.82
Solid	*L. casei*	37	7	8.27
Solid	*B. subtilis*	30	7	10.57
Solid	*B. subtilis*	37	7	9.96
Solid	*S. cerevisiae*	30	7	7.93
Solid	*S. cerevisiae*	37	7	7.55
Solid	*A. oryzae*	25	7	6.83
Solid	*A. oryzae*	30	7	7.77
Control group				6.30

from *L. casei* and *B. subtilis* conditions. The *L. casei*, *B. subtilis* and *S. cerevisiae* fermented extracts exhibited higher yields at 30°C than at 37°C, while *A. oryzae* extract showed the opposite trend. Particularly, yields of *L. casei* and *B. subtilis* fermented extracts ranged from 8.27-10.57%, while *S. cerevisiae* and *A. oryzae* fermented extracts had yields of 6.83-7.93%, all surpassing the control group at 6.3%. In liquid fermentation conditions, yields of *L. casei*, *B. subtilis* and *S. cerevisiae* fermented extracts were 20.77, 9.32 and 4.18%, respectively. Notably, extracts from *L. casei* and *B. subtilis* fermentations showed increased yields compared to the control group (Table 3).

Variations in physiologically active compound content according to fermentation conditions

Calibration curve: In Table 4, calibration curves for the quantification of apigenin, luteolin, nodakenin and scopoletin within the samples were presented. The R^2 values for the calibration curves of apigenin, luteolin, nodakenin and scopoletin were all above 0.99.

Apigenin content: The examination of apigenin content variations in response to *A. dahurica* fermentation conditions yielded noteworthy results. Specifically, when subjected to liquid fermentation, conditions *B. subtilis* and *S. cerevisiae* induced a considerable increase in apigenin content, surpassing the control value of 0.1481 µg/mg. Furthermore, the apigenin content resulting from the utilization of condition *S. cerevisiae* for fermentation stood notably higher at 0.5172 µg/mg compared to condition *B. subtilis*. However, apigenin remained undetectable under all other experimental conditions (Table 5).

Upon analysis of apigenin content following liquid fermentation of the *E. splendens*, conditions *B. subtilis* and *S. cerevisiae* once again exhibited a substantial augmentation in apigenin content relative to the control, registering at 0.2434 µg/mg. Additionally, when *S. cerevisiae* was employed for fermentation, the apigenin content significantly outstripped that of condition *B. subtilis*, with a remarkable value of 1.5069 µg/mg. In the context of solid-state fermentation, apigenin content underwent a notable increase when conditions *S. cerevisiae* and *A. oryzae* were employed, again surpassing the control value of 0.2434 µg/mg. Particularly noteworthy is the higher apigenin content observed when utilizing condition *A. oryzae*. Additionally, a discernible trend emerged, indicating that lower temperatures were associated with increased apigenin content. Specifically, when employing condition *A. oryzae* for fermentation at a temperature of 25°C, apigenin content reached its zenith at 1.4904 µg/mg. Nevertheless, apigenin was not detectable under all other experimental conditions (Table 5).

Fermenting with *S. cerevisiae* at 30°C for one day presented the highest apigenin content. Luteolin, a type of flavonoid, can be synthesized through metabolic processes by *S. cerevisiae*[34,35].

Luteolin content: The assessment of luteolin content following the fermentation of *A. dahurica* reveals intriguing trends. Across all conditions, luteolin content consistently exceeded the baseline luteolin content of 1.0390 µg/mg. In the context of liquid fermentation, luteolin content followed the order *S. cerevisiae*, *B. subtilis* and *L. casei*, with the highest content observed when employing condition *S. cerevisiae*, reaching a maximum of 4.9428 µg/mg. For solid-state fermentation, luteolin content displayed a trend of increasing levels in the order of *A. oryzae, S. cerevisiae*, *L. casei* and *B. subtilis*, with *L. casei* and *B. subtilis* exhibiting similar content levels. Notably, *L. casei*, *B. subtilis* and *S. cerevisiae* displayed a positive correlation between lower temperatures and higher luteolin content, while for condition *A. oryzae*, higher temperatures were associated with increased luteolin content. Consequently, the highest luteolin content of 3.7739 µg/mg was achieved when utilizing condition *A. oryzae* at 30°C (Table 6).

In the control group of *E. splendens*, luteolin was detected at a level of 1.4937 µg/mg. In liquid fermentation, luteolin was only detected when employing condition *S. cerevisiae*, with a content level of 1.1241 µg/mg. In solid-state fermentation, luteolin was detected exclusively when condition *A. oryzae* was utilized and notably, at a higher level when fermented at 25°C compared to 30°C. However, it is worth mentioning that these levels remained comparatively lower when compared to the control group (Table 6).

The optimum fermentation conditions for luteolin were found to be fermenting with *S. cerevisiae* at 30°C for one day. Like apigenin, luteolin is a type of flavonoid and the increase is interpreted to be due to *S. cerevisiae* enhancing flavonoid production during metabolic processes[34,35].

Nodakenin content: The nodakenin level in the control group of *A. dahurica* was determined to be 1.8809 µg/mg. However, depending on the fermentation conditions, the nodakenin content exhibited fluctuations, both decreasing and increasing. In the context of liquid fermentation, the order of nodakenin content was *S. cerevisiae*, *B. subtilis* and *L. casei*, with the highest content observed when condition *S. cerevisiae* was utilized, reaching a level of 2.4979 µg/mg, surpassing that of the control. For solid-state fermentation, there was a tendency for nodakenin content to increase in the order of *S. cerevisiae*, *A. oryzae*, *B. subtilis* and *L. casei*. In conditions *L. casei*, *B. subtilis* and *A. oryzae*, higher temperatures were associated with increased nodakenin content, whereas for condition *S. cerevisiae*, lower temperatures yielded higher nodakenin content. Accordingly, the highest nodakenin content of 1.6731 µg/mg was achieved when employing condition *S. cerevisiae* at 30°C, although it remained lower than the control (Table 7).

The nodakenin content in the control group of *E. splendens* registered at 0.6279 µg/mg. Similarly, the nodakenin content exhibited fluctuations based on fermentation conditions, both decreasing and increasing. In the case of liquid fermentation, nodakenin was not detected

Table 4: Calibration curve of apigenin, luteolin, nodakenin and scopoletin

Substance	Calibration curve	R^2
Apigenin	y = 0.0739x+0.5478	0.9988
Luteolin	y = 0.1097x+2.3408	0.9914
Nodakenin	y = 0.2520x-0.0355	1.0000
Scopoletin	y = 0.0429x+0.0590	0.9998

Table 5: Variations in apigenin content in *Angelica dahurica* and *Elsholtzia splendens* based on fermentation conditions

		Fermentation conditions		
State	Strains	Temperature (°C)	Time (days)	Apigenin content (μg/mg)
A. dahurica				
Liquid	*L. casei*	30	1	-
Liquid	*B. subtilis*	30	1	0.1740
Liquid	*S. cerevisiae*	30	1	0.5172
Solid	*L. casei*	30	7	-
Solid	*L. casei*	37	7	-
Solid	*B. subtilis*	30	7	-
Solid	*B. subtilis*	37	7	-
Solid	*S. cerevisiae*	30	7	-
Solid	*S. cerevisiae*	37	7	-
Solid	*A. oryzae*	25	7	-
Solid	*A. oryzae*	30	7	-
Control group				0.1481
E. splendens				
Liquid	*L. casei*	30	1	-
Liquid	*B. subtilis*	30	1	0.3576
Liquid	*S. cerevisiae*	30	1	1.5069
Solid	*L. casei*	30	7	-
Solid	*L. casei*	37	7	-
Solid	*B. subtilis*	30	7	-
Solid	*B. subtilis*	37	7	-
Solid	*S. cerevisiae*	30	7	0.2963
Solid	*S. cerevisiae*	37	7	-
Solid	*A. oryzae*	25	7	1.5052
Solid	*A. oryzae*	30	7	1.1400
Control group				0.2434

and it was only detected when employing solid-state fermentation with condition *A. oryzae*. Notably, higher temperatures were associated with higher nodakenin content. Consequently, when utilizing condition *A. oryzae* for fermentation at 30°C, the nodakenin content reached its highest level of 0.6487 μg/mg, surpassing that of the control group (Table 7).

In the majority of the fermentation processes conducted in this study, there were decreases in the nodakenin content. Even when an increase occurred, it was relatively modest. However, in studies by Lee *et al*.[36] and Kim *et al*.[37], it was observed that fermentation led to not only a reduction in nodakenin content but also an increase in nodakenetin content. Therefore, additional investigations into the change of nodakenetin content are considered necessary.

Scopoletin content: The scopoletin was identified at a concentration of 0.1540 μg/mg in the control group of *A. dahurica*. Liquid fermentation only yielded scopoletin when condition *S. cerevisiae* was employed, with a content level of 0.3958 μg/mg, surpassing that of the control group. Similarly, solid-state fermentation only resulted in the detection of scopoletin when condition *S. cerevisiae* was used, with content levels exceeding those of the control. Furthermore, scopoletin content was higher at 30°C compared to 37°C (Table 8).

In the control group of *E. splendens*, scopoletin was detected at a level of 0.2495 μg/mg and its content exhibited sensitivity to changes in fermentation conditions, resulting in both increases and decreases. Scopoletin was not detected during liquid fermentation. However, during solid-state fermentation, it was only detected when conditions *B. subtilis*, *S. cerevisiae* and *A. oryzae* were used. Notably, when conditions *S. cerevisiae* and *A. oryzae* were employed, the scopoletin content exceeded that of the control group. The order of scopoletin content was *A. oryzae*, *S. cerevisiae* and *B. subtilis*, with *B. subtilis* showing a positive correlation with higher temperatures,

Table 6: Variations in luteolin content in *Angelica dahurica* and *Elsholtzia splendens* based on fermentation conditions

		Fermentation conditions		
State	Strains	Temperature (°C)	Time (days)	Luteolin content (μg/mg)
A. dahurica				
Liquid	*L. casei*	30	1	1.5602
Liquid	*B. subtilis*	30	1	1.8667
Liquid	*S. cerevisiae*	30	1	4.9428
Solid	*L. casei*	30	7	1.8412
Solid	*L. casei*	37	7	1.7609
Solid	*B. subtilis*	30	7	1.8134
Solid	*B. subtilis*	37	7	1.7523
Solid	*S. cerevisiae*	30	7	3.1318
Solid	*S. cerevisiae*	37	7	2.5642
Solid	*A. oryzae*	25	7	2.8459
Solid	*A. oryzae*	30	7	3.7739
Control group				1.0390
E. splendens				
Liquid	*L. casei*	30	1	-
Liquid	*B. subtilis*	30	1	-
Liquid	*S. cerevisiae*	30	1	1.1241
Solid	*L. casei*	30	7	-
Solid	*L. casei*	37	7	-
Solid	*B. subtilis*	30	7	-
Solid	*B. subtilis*	37	7	-
Solid	*S. cerevisiae*	30	7	-
Solid	*S. cerevisiae*	37	7	-
Solid	*A. oryzae*	25	7	1.2930
Solid	*A. oryzae*	30	7	0.9898
Control group				1.4937

Table 7: Variations in nodakenin content in *Angelica dahurica* and *Elsholtzia splendens* based on fermentation conditions

		Fermentation conditions		
State	Strains	Temperature (°C)	Time (days)	Nodakenin content (μg/mg)
A. dahurica				
Liquid	*L. casei*	30	1	0.6583
Liquid	*B. subtilis*	30	1	0.9193
Liquid	*S. cerevisiae*	30	1	2.4979
Solid	*L. casei*	30	7	0.7373
Solid	*L. casei*	37	7	0.7983
Solid	*B. subtilis*	30	7	0.7424
Solid	*B. subtilis*	37	7	0.9154
Solid	*S. cerevisiae*	30	7	1.6731
Solid	*S. cerevisiae*	37	7	1.3946
Solid	*A. oryzae*	25	7	1.1907
Solid	*A. oryzae*	30	7	1.6624
Control group				1.8809
E. splendens				
Liquid	*L. casei*	30	1	-
Liquid	*B. subtilis*	30	1	-
Liquid	*S. cerevisiae*	30	1	-
Solid	*L. casei*	30	7	-
Solid	*L. casei*	37	7	-
Solid	*B. subtilis*	30	7	-
Solid	*B. subtilis*	37	7	-
Solid	*S. cerevisiae*	30	7	-
Solid	*S. cerevisiae*	37	7	-
Solid	*A. oryzae*	25	7	0.5166
Solid	*A. oryzae*	30	7	0.6487
Control group				0.6279

Table 8: Variations in scopoletin content in *Angelica dahurica* and *Elsholtzia splendens* based on fermentation conditions

		Fermentation conditions		
State	Strains	Temperature (°C)	Time (days)	Scopoletin content (μg/mg)
A. dahurica				
Liquid	*L. casei*	30	1	-
Liquid	*B. subtilis*	30	1	-
Liquid	*S. cerevisiae*	30	1	0.3958
Solid	*L. casei*	30	7	-
Solid	*L. casei*	37	7	-
Solid	*B. subtilis*	30	7	-
Solid	*B. subtilis*	37	7	-
Solid	*S. cerevisiae*	30	7	0.2854
Solid	*S. cerevisiae*	37	7	0.2206
Solid	*A. oryzae*	25	7	-
Solid	*A. oryzae*	30	7	-
Control group				0.1540
E. splendens				
Liquid	*L. casei*	30	1	-
Liquid	*B. subtilis*	30	1	-
Liquid	*S. cerevisiae*	30	1	-
Solid	*L. casei*	30	7	-
Solid	*L. casei*	37	7	-
Solid	*B. subtilis*	30	7	0.2136
Solid	*B. subtilis*	37	7	0.2198
Solid	*S. cerevisiae*	30	7	0.2745
Solid	*S. cerevisiae*	37	7	0.2503
Solid	*A. oryzae*	25	7	0.3903
Solid	*A. oryzae*	30	7	0.3517
Control group				0.2495

while *S. cerevisiae* and *A. oryzae* demonstrated higher content levels at lower temperatures. Consequently, the highest scopoletin content of 0.3903 μg/mg was achieved when utilizing condition *A. oryzae* for fermentation at 25°C, significantly surpassing that of the control group (Table 8).

According to Zhao *et al*.[38], Choi *et al*.[39] and Bayoumi *et al*.[40], scopoletin is a compound whose biosynthetic pathway is not clearly identified despite various advantages such as antioxidant and anti-inflammatory. Therefore, it is notable that the content of scopoletin increased when fermenting *A. dahurica* with *S. cerevisiae* and *E. splendens* with *A. oryzae*. Furthermore, it is considered that further research is essential to clarify the component changes in components through the analysis of other coumarin-based compounds in accordance with the metabolic process.

This study provides a fundamental understanding of physiologically active compound and a further study is needed. Furthermore, additional experiments and clinical research are necessary to investigate the safety and efficacy of the fermented products.

CONCLUSION

In this study, we investigated the changes in the contents of physiologically active compounds, namely apigenin, luteolin, nodakenin and scopoletin, in *Angelica dahurica* and *Elsholtzia splendens* under various fermentation conditions. The results of this research provide insights into fermentation conditions that enhance the levels of beneficial physiologically active compounds in *Angelica dahurica* and *Elsholtzia splendens*. Therefore, the application of these findings were proposed as a valuable materials in various fields such as cosmetics, food and pharmaceuticals, where these compounds can be utilized.

SIGNIFICANCE STATEMENT

There is limited research on fermentation-related studies on natural products. This study compared and analyzed the variations in the content of active ingredients when different extraction methods, such as fermentation microorganisms and temperature, applying to *Angelica dahurica* and *Elsholtzia splendens*. The results indicated a trend of increased content observed in liquid fermentation when *Saccharomyces cerevisiae* was used and in solid-state fermentation when *Saccharomyces cerevisiae* and *Aspergillus oryzae* were used. This result suggests the potential applications of these findings in cosmetics, food, pharmaceuticals.

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