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Enhancement of bioactive compounds through bioconversion of *Oenanthe javanica* using *Lactiplantibacillus plantarum*

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Abstract

The purpose of this study is to investigate the effect of bioconverted *Oenanthe javanica* extract (BOE) using *Lactiplantibacillus plantarum* SM4 on production of bioactive compounds. Response surface methodology was used to determine the optimal ultrasound-assisted extraction conditions including the extraction time, extraction temperature, and ethanol concentration for the enhancement of bioactive compounds production. The predicted optimum UAE conditions were extraction time of 26.0 min, extraction temperature of 92.8 °C, and 59.9% ethanol, respectively, and 5.14 mg GAE/g DM of TPC and 0.59 mg QE/g DM of TFC were produced under the optimum condition. Then, *O. javanica* extraction (OJE) has been bioconverted by using *L. plantarum* SM4 to increase the bioactive compounds. In HPLC-mass spectrometry analyses, higher concentration of *p*-coumaric acid was identified in BOE than in OJE, and chlorogenic acid in OJE was converted to quinic acid, confirming an increase in bioactive compounds by bioconversion. Thus, we have concluded that optimization of UAE and the BOE is an effective process for enhancement bioactive compounds from *O. Javanica* and this finding provides a scientific basis of bioactive compounds in natural product can be utilized as food, cosmetics, and pharmaceutical materials from BOE.

Keywords: *Oenanthe japonica*; *Lactiplantibacillus plantarum*; ultrasound assisted extract; bioconversion; *p*-coumaric acid.

Practical Application: *Oenanthe javanica* is widely distributed across temperate and tropical Asia. It is a highly useful functional food ingredient because it can efficiently lower the blood pressure and cholesterol levels, antipyretic, detoxification, and ameliorate small intestine-associated diseases. So this study is to investigate the effect of bioconverted Oenanthe javanica extract (BOE) using Lactiplantibacillus plantarum SM4 on production of bioactive compounds.

1 Introduction

O_2 and CO_2 are the primary gaseous substrate and product of oxidative phosphorylation in respiring organisms, respectively. However, approximately 2% of the O_2 is converted into reactive oxygen species (ROS) during oxidative adenosine triphosphate (ATP) production, wherein O_2 is reduced to water in the mitochondria (Waypa et al., 2016; Snezhkina et al., 2019). ROS can cause a variety of protein modifications, including metal-catalyzed carbonylation, oxidation of aromatic and sulfur-containing amino acid residues, oxidation of the protein backbone and protein fragmentation due to oxidation of amino acid side chains as well as protein backbones. ROS may induce the mitochondrial permeability transition pore (mPTP) to open within individual mitochondria in cell systems (Muller et al., 2018; Gao et al., 2022). The opening of this pore is a mitochondrial response to oxidative stress, resulting in an amplified ROS signal, which may cause cell death depending on the ROS level (Korge et al., 2011; Li et al., 2015). Besides the mitochondria, ROS can be released into the cell through nicotinamide adenine dinucleotide (NADH) oxidase, cyclooxygenase, nitric oxide synthase (NOS), the mitochondrial electron transport system, and cellular injury (Spolarics, 1996). ROS are highly unstable and damage cells by attacking intracellular proteins, nucleic acids, and cell membranes (Min et al., 2022). To maintain homeostasis, they are mostly eliminated by defense mechanisms, such as the antioxidant defense system, which includes glutathione peroxidase (GPx) catalase (CAT), and superoxide dismutase (SOD) (Wang, 2022; Jóźwiak & Politycka, 2019). However, when the rate of ROS generation exceeds that of elimination, homeostasis is perturbed, resulting in cellular damage and the loss of normal function, which can cause diseases like arteriosclerosis and cardiovascular disease as well as accelerated aging (Wilmes et al., 2020; Hao et al., 2022). Therefore, in-depth research on various methods for efficient ROS regulation is ongoing, especially focusing on natural bioactive compounds or secondary metabolites with antioxidant activity, which can activate antioxidant enzymes (Donadio et al., 2021; Setyorini & Antarlina, 2022).

Secondary metabolites can be extracted from natural compounds using conventional methods, such as hot water, Soxhlet and supercritical extraction (Hartonen et al., 2007). However, the yield often decreases due to the decomposition of bioactive compounds

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under long extraction times and high temperatures (Mokrzycki et al., 2020; Ren et al., 2022). On the other hand, bioconversion using fermentation exploits the substrate specificity of enzymes that induce hydrolyzation or structural changes in macromolecules present in the natural compounds, thereby increasing the yield of the bioconverted products or the production of new compounds as reactants or hydrolyzed products (Gulsunoglu-Konuskan & Kilic-Akyilmaz, 2022). In addition, microbe-derived enzymes produced during fermentation, such as β-glycosidase and β-glucuronidase, are known to enhance their bioactivity by increasing the hydrolysis of glycosidic bonds to terminal non-reducing residues in β-D-glucosides and oligosaccharides that are bonded within the plant cellular tissues to small molecules (Kim et al., 2022; Kumari et al., 2021; Kang et al., 2020). The WHO and FAO considered probiotics as 'live microorganisms when administered in adequate amounts confer a health benefit on the host' (Zendebooodi et al., 2020). Among the many beneficial effects of probiotic bacteria, there is a lot of interest and research going on that probiotics can improve the nutritional value of food. Bioconversion is mainly made up of fermentation using probiotics, so it is possible to enhance the nutritional and physiological values through enzymatic conversion of raw materials of food. Especially, in the case of *Lactiplantibacillus plantarum* used in this study, bioactivity such as antioxidant, anti-inflammation, and anti-diabetes was reported, and recent studies have reported cancer cell death through *MAPK* signaling pathways and suppression of metastasis through *MMP-1/3* inhibition (Zommara et al., 2022; Shukla et al., 2022). Therefore, research on improving physiological activity and enhancing nutritional value of food products through bioconversion using *Lactiplantibacillus plantarum* in natural products continues.

Oenanthe javanica, a plant originating from East Asia, is widely distributed across temperate and tropical Asia, including Korea, Japan, and China. It is used as food in the regions of Southeast Asia, including Taipei, Malaysia, and India (Sung et al., 2010; Kim, 2012). *O. javanica* is rich in vitamin B_1, B_2, Zn, and Ca, and β-carotenoid. Nutritionally, it is a highly useful functional food ingredient because it can efficiently lower the blood pressure and cholesterol levels, antipyretic, detoxification, and ameliorate small intestine-associated diseases (Won et al., 2015; Jo et al., 2008). In addition, as *O. javanica* is known to contain many polyphenols such as kaempferol and quercetin, a consumption of *O. javanica* has been reported to be effective in reducing insulin resistance and body fat rate in the elderly (Kim et al., 2017).

In this study, we optimized the conditions for extracting bioactive compounds using statistically based optimization and converted these compounds using enzymes produced via fermentation. In addition, the total polyphenol and flavonoid contents were measured and the main compounds in bioconverted *O. javanica extract* (BOE) were identified to assess the potential of the extract as an ingredient of pharmaceuticals and functional foods.

2 Results and discussion

2.1 Optimization of TPC and TFC extraction condition

The TPC and TFC were measured to determine the optimal UAE conditions for extracting polyphenols from *O. javanica* extract (OJE). The 12th (30.0 min, 94.0 °C, and 50.0% ethanol) and 7th (15.0 min, 80.0 °C, and 80.0% ethanol) experimental groups showed maximum values of 7.27 mg GAE/g DM and 0.68 mg QE/g DM, respectively (Table 1). To further evaluate the experimental model, we analyzed the coefficient of determination (R^2), which represents the extent of fit between the experimental and predicted values: when R^2 is close to 1, the fit is better. The R^2 values for the quadratic regression equation functions of TPC and TFC were 0.9194 and 0.8760, respectively, based on the results of the 17 conditions according to the central composite design (CCD), confirming the suitability of the quadratic regression equations (Table 2). Analysis of variance (ANOVA) was used to evaluate the significance of each experimental model variable and the identified *p*-value was within 5% for both TPC and TFC. The extraction temperature ($p = 0.0056$) was found to have the highest effect on TPC, whereas ethanol concentration ($p = 0.0004$) had the highest effect on TFC (Table 3).

The effect of an independent variable on TPC and TFC was shown in a perturbation plot with the two variables fixed on the center point (Figure 1). The range of TPC and TFC increased with increasing extraction temperatures and ethanol concentrations, respectively, further confirming that these two variables are the most prominent for optimizing polyphenol extraction from OJE. When the interactions between independent variables were analyzed, we found that TPC increased with ethanol concentration, decreasing at 48.4%, and temperature had a proportional relationship with TPC (Figure 2A, B). Min et al. (2010), reported similar results, stating that the optimal ethanol concentration for polyphenol extraction from jujube leaf was 45.0%. Furthermore, Kim et al. (2017), demonstrated that that the TPC of *Gynostemma pentaphyllum* increased as the combined polyphenol was converted to free polyphenol because of the increased molecular motion caused by the increase in

Table 1. Experimental UAE conditions of CCD with three independent variables for extraction of bioactive compounds from *O. javanica*.

Run No	Extraction conditions			TPC (mg GAE/g DM)	TFC (mg QE/g DM)
	X_1	X_2	X_3		
1	15.0	40.0	20.0	4.917	0.399
2	45.0	40.0	20.0	4.591	0.387
3	15.0	80.0	20.0	5.671	0.478
4	45.0	80.0	20.0	5.277	0.345
5	15.0	40.0	80.0	3.983	0.571
6	45.0	40.0	80.0	4.651	0.599
7	15.0	80.0	80.0	4.617	0.677
8	45.0	80.0	80.0	5.054	0.656
9	5.0	60.0	50.0	5.638	0.410
10	55.0	60.0	50.0	5.694	0.399
11	30.0	26.0	50.0	5.131	0.394
12	30.0	94.0	50.0	7.265	0.534
13	30.0	60.0	0.0	5.031	0.189
14	30.0	60.0	99.5	2.968	0.615
15	30.0	60.0	50.0	6.105	0.549
16	30.0	60.0	50.0	6.161	0.545
17	30.0	60.0	50.0	6.162	0.556

Experimental data on TPC and TFC of OJE under 17 sets of extraction conditions based on CCD. TPC: total polyphenol content, TFC: total flavonoid content.

Table 2. Polynomial equations generated by CCD for the prediction of UAE optimum condition from *O. javanica*.

Responses	Polynomial equations	R^2	*p value*
TPC (mg GAE/g DM)	$Y_{(TPC)} = 6.17 + 0.037X_1 + 0.44X_2 - 0.42X_3 - 0.037X_1X_2 + 0.23X_1X_3 - 0.050X_2X_3 - 0.25X_1^2 - 0.061X_2^2 - 0.87X_3^2$	0.9194	0.0043
TFC (mg QE/g DM)	$Y_{(TFC)} = 0.54 - 0.011X_1 + 0.032X_2 + 0.12X_3 - 0.021X_1X_2 - 0.019X_1X_3 + 0.016X_2X_3 - 0.029X_1^2 - 8.38210^{-3}X_2^2 - 0.030X_3^2$	0.8760	0.0202

A negative coefficient in each equation represents an antagonistic effect of the variables and a positive coefficient represents a synergistic effect of the variables.

Table 3. ANOVA of the experimental results of CCD for statistical evaluation of polynomial equations of TPC and TFC.

	TPC (mg GAE/g DM)			TFC (mg QE/g DM)		
	Sum of Squares	*F* value	*p value*	Sum of Squares	*F* value	*p value*
Model	13.97	8.94	0.0043	0.23	5.23	0.0202
X_1	0.019	0.11	0.7529	1.76010^{-3}	0.36	0.5672
X_2	2.69	15.49	0.0056	0.014	2.85	0.1353
X_3	2.37	13.63	0.0077	0.19	38.76	0.0004
X_1X_2	0.011	0.064	0.8071	3.61210^{-3}	0.74	0.4182
X_1X_3	0.42	2.40	0.1655	2.88810^{-3}	0.59	0.4671
X_2X_3	0.020	0.12	0.7425	1.98510^{-3}	0.41	0.5441
X_1^2	0.72	4.12	0.0818	9.60010^{-3}	1.97	0.2037
X_2^2	0.042	0.24	0.6362	8.09510^{-4}	0.17	0.6961
X_3^2	8.09	46.56	0.0002	9.85210^{-3}	2.02	0.1985

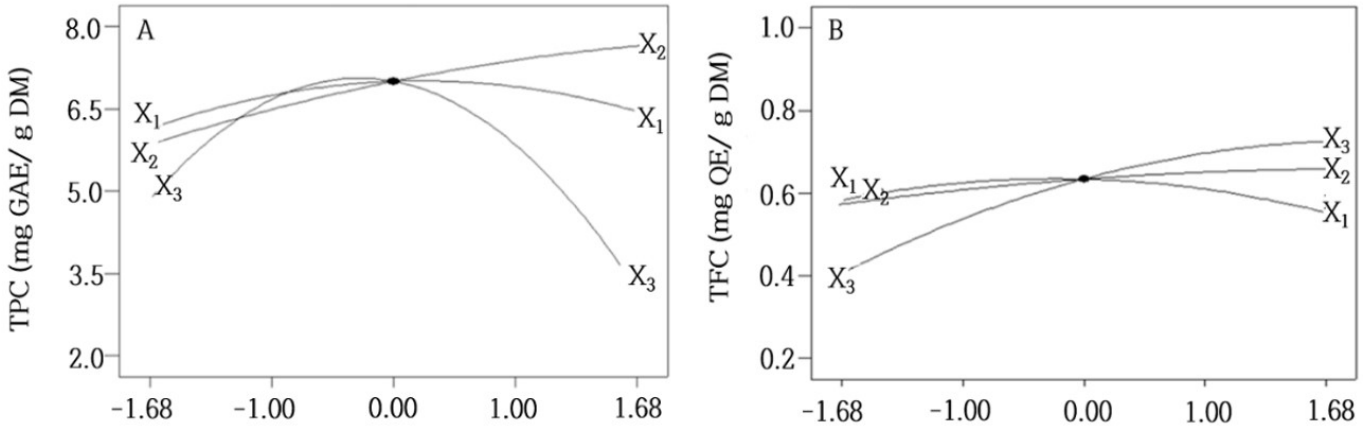

Figure 1. Perturbation plots for the evaluation of the effect of (X_1) extraction time (X_2) extraction temperature, and (X_3) ethanol concentration on the TPC and TFC of OJE.; OJE: *O. Javanica* extract. (A) Perturbation plots for TPC of O. Javanica; (B) Perturbation plots for TFC of O. Javanica.

extraction temperature (Kim & Kim, 2019; Yeom et al., 2022). On the other hand, as the ethanol concentration increased, TFC correspondingly increased, irrespective of the extraction time or temperature (Figure 2C, D). The optimization of ethanol concentration is mandatory for increased TPC and TFC; however, some polyphenols might decompose at high temperatures. Hence, maximum polyphenol yield can be obtained by optimizing both the ethanol concentration and temperature. The maximum values of TPC and TFC observed were found to match those predicted by CCD. The TPC was predicted to 6.82 mg GAE/g DM at an extraction time of 27.1 min, extraction temperature of 94.0 °C, and 40.5% ethanol, while the TFC was predicted to be 0.67 mg QE/g DM at an extraction time of 25.9 min, 93.8 °C extraction temperature, and 99.5% ethanol.

2.2 Optimization of UAE condition

Statistical optimization using CCD was conducted to establish optimum UAE conditions for the extraction of polyphenols from OJE. The range of optimum UAE conditions was predicted by superimposing the individual response surface of TPC and TFC (Figure 3). The range of independent variables for the extraction time, temperature, and ethanol concentration was 5.0-55.0 min, 26.0-94.0 °C, and 0.0-99.5%. Further, by setting points where the time and temperature were minimal, considering the economic feasibility of the industrial extraction process, the optimized UAE conditions for all the dependent variables were simultaneously derived as 26.0 min, 92.8 °C, and 59.9% ethanol. Using these conditions, the TPC

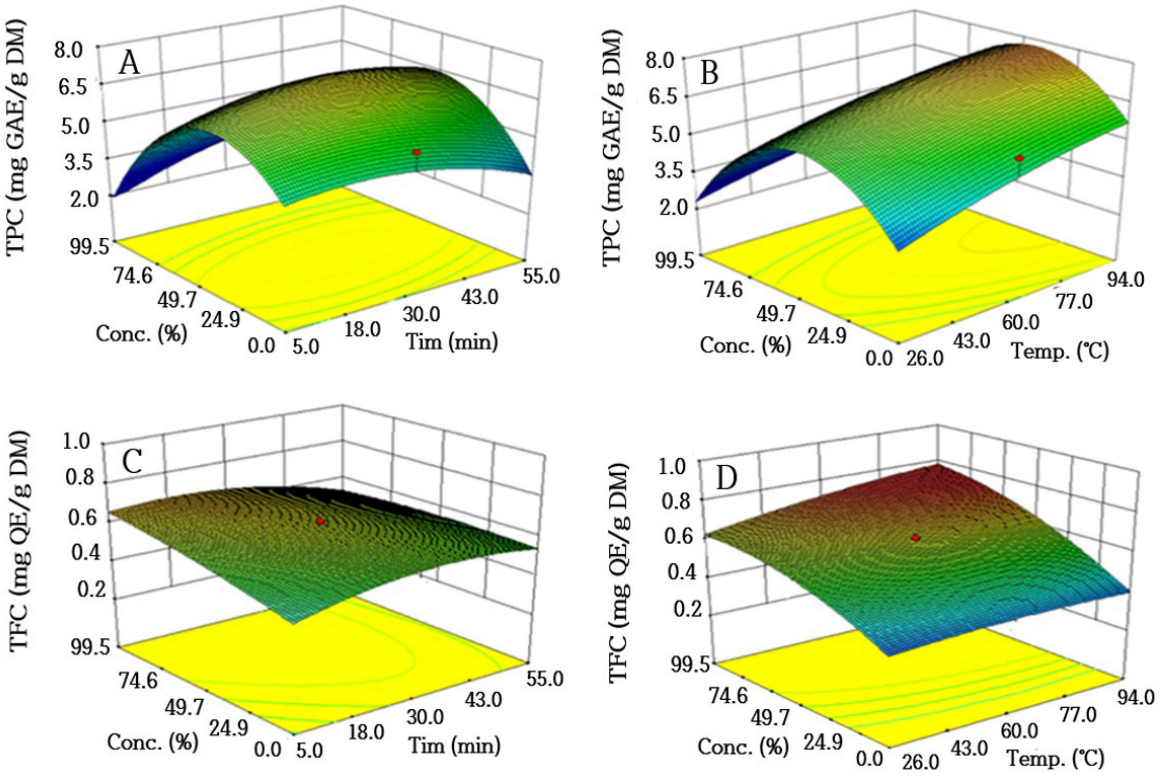

Figure 2. Response surface plots showing the interactive effect of extraction time, extraction temperature, and ethanol concentration interaction on TPC and TFC. (A) TPC as a function of extraction time and ethanol concentration; (B) extraction temperature and ethanol concentration; (C) TFC as a function of extraction time and ethanol concentration; (D) extraction temperature and ethanol concentration.

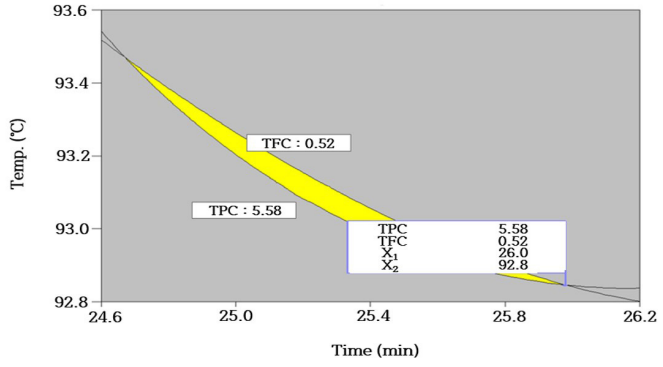

Figure 3. Overlay contour map for the simultaneous maximization of three variables for the maximization TPC and TFC. Ethanol concentration was fixed at the optimum level of 59.9%.

and TFC predicted were 5.14 ± 0.31 mg GAE/g DM and 0.59 ± 0.08 mg QE/g DM, respectively. The experimental values obtained TPC 5.21 ± 0.41 mg GAE/g DM and 0.56 ± 0.06 mg QE/g DM, respectively, thereby confirming the effectiveness of the statistical optimization and validity of the CCD prediction.

2.3 Analysis of main compound

According to a previous study by Gam et al. (2022), chlorogenic acid was bioconverted into caffeic acid as evaluated via HPLC, and they intended to confirm it using HPLC-MS/MS. We compared the distribution of molecular weights by HPLC-MS/MS to determine the main bioactive compounds in OJE and BOE. In OJE, ion peaks with mass to charge ration (m/z) values of 190 and 353 were separated at an retention time (RT) of 7.4 min, presumably corresponding to chlorogenic acid (m/z = 354.3) without a caffeoyl group (m/z = 163.0) and intact chlorogenic acid, respectively. Chlorogenic acid is formed by the esterification of quinic acid and caffeic acid. Caffeic acid (m/z = 178.7) and *p*-coumaric acid (m/z = 165.0) were confirmed at RT 8.7 and 16.4 min, respectively. Therefore, the extraction of polyphenols, such as chlorogenic acid, caffeic acid, and *p*-coumaric acids, was confirmed.

BOE was resolved into three peaks with m/z values of 191.1, 173.0, and 85.9 at an RT of 12.1 min. The first two correspond to MS fragments of quinic acid while the third is quinic acid with an H_20 molecule detached. Considering that quinic acid was not detected among the compounds extracted from OJE, new useful substances were produced via bioconversion through fermentation using *Lactiplantibacillus plantarum* SM4 in this study (Figures 4, 5).

In plants, quinic acid exists in the form of CA due to esterification with caffeic acid. Considering that chlorogenic acid was detected in OJE, but not in BOE, the quinic acid increased in the form of chlorogenic acid probably decomposed by bioconversion using *L. plantarum* SM4. Similarly, Santana-Gálvez et al. (2017), reported that chlorogenic acid disintegrated

simultaneously into caffeic acid and quinic acid during bioconversion by *L. plantarum* (Lee et al., 2014). Caffeic acid and quinic acid have been reported to have beneficial effects on various diseases, such as obesity, neurodegenerative, and cardiovascular disease (Bao et al., 2021). In a similar study, Lee et al. (2014), reported that bioconversion of the bioactive compounds in citrus fruits, such as the flavonoids of naringin, neohesperidin, and hesperidin, into other compounds increased their antibacterial and anticancer effects. Accordingly, polyphenols contained in OJE were confirmed to be converted into new, low-molecular compounds like QA through bioconversion by *L. plantarum* SM4.

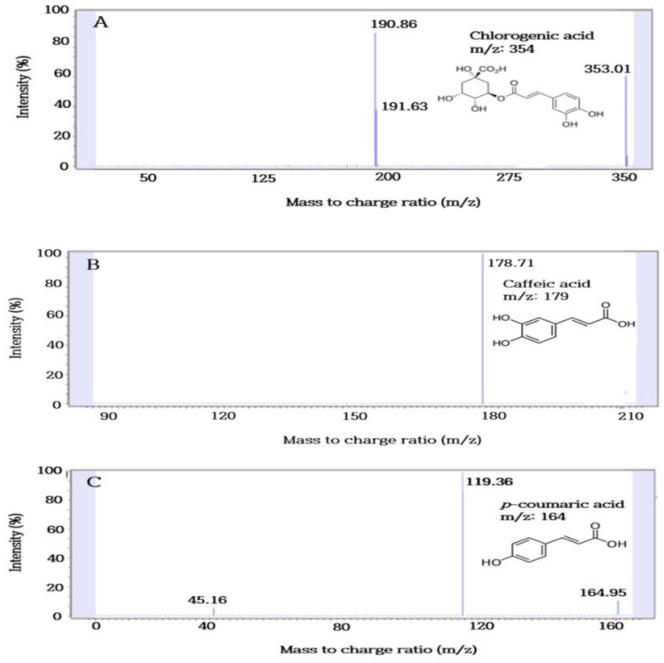

Figure 4. Spectra of HPLC-MS/MS fragmentation patterns of polyphenols isolated from OJE. (A) chlorogenic acid, (B) caffeic acid, and (C) *p*-coumaric acid.

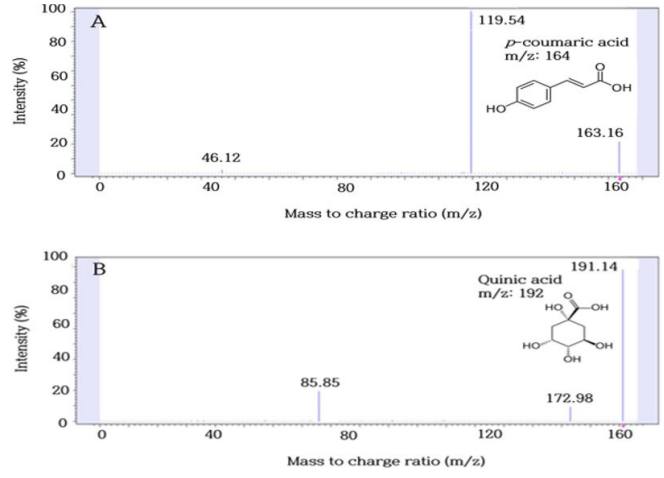

Figure 5. Spectra of HPLC-MS/MS fragmentation patterns of polyphenols isolated from BOE. (A) *p*-coumaric acid, (B) quinic acid.

3 Materials and methods

3.1 Materials and reagents

The *O. Javanica* used in this experiment was purchased from the Hana agricultural corporation in Yangsan, Gyeongsangnam-do in 2020. Ethanol (Duksan Sci. Co., Gapyoung, Korea, 99.5%) and distilled water (DW) were used as the extraction solvents. Gallic acid and quercetin, the standard substances used in the analysis of total polyphenol and flavonoid contents, were purchased from Sigma-aldrich Co. (MO, USA). MRS medium for *L. plantarum* SM4 culture and extract bioconversion was purchased from BD Difco (Sparks, MD, USA). The standard substances used in HPLC analysis, such as chlorogenic acid, caffeic acid, *p*-coumaric acid, rutin, quercetin, and isorhamnetin with purity of 99.8% or higher were purchased from Sigma-aldrich Co. Acetonitrile, acetic acid, and formic acid used in HPLC for quantitative and qualitative analysis were purchased from Sigma-aldrich Co. in HPLC grade.

3.2 Experimental design

For the optimization of polyphenol extraction conditions from *O. Javanica*, CCD using Design-Expert software (v.8.0, stat-Ease, Minneapolis, USA) was applied. The independent variables were selected as extraction time (X_1), extraction temperature (X_2), and ethanol concentration (X_3), which are the principal influence factors of ultrasound-assisted extraction (UAE) based on previous research. The variance of the selected independent variables was coded in five levels (-1.68, -1, 0, 1, 1.68), and extracted according to 17 experimental conditions (Table 4). The dependent variables affected by these independent variables were set up as total polyphenol content (TPC) and total flavonoid content (TFC). The quadratic regression equation derived from each experimental value based on each of the extraction conditions are as follows Equation 1.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_{12} + \beta_{22} X_{22} + \beta_{33} X_{32} + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

Level of each variable was established based on preliminary experiments by on one-factor-at-a-time method. The distance of the axial points from the center point was ± 1.68.

3.3 Ultrasound-assisted extraction

The *O. javanica* leaves and stem was washed and dried using the dry oven (VS-1202D4N, Vision Bionex, Buchoen, Korea) for 48 hr at 60 °C and powdered below 40 mesh (0.4 mm) using a grinder (HMF-3000S, Hanil Co., Wonju, Korea). The extraction solvent composed of ethanol (95.0%) and distilled water (DW)

Table 4. Independent variables and their coded and actual values used for optimization of UAE conditions of *O. javanica.*

X_i	Independent variables	Coded levels				
		-1.68	-1	0	+1	+1.68
X_1	Extraction time (min)	5.0	15.0	30.0	45.0	55.0
X_2	Extraction temperature (°C)	26.0	40.0	60.0	80.0	94.0
X_3	Ethanol concentration (% v/v)	0.0	20.0	50.0	80.0	99.5

was mixed with *O. Javanica* at a 1:20 solid/liquid and extracted in ultrasound extractor (SD-D250H, Sungdong Co., Hwaseong, Korea) while performing different time and temperatures in 40 kHz and 200 W conditions. After UAE, the extract was supernatant separated at 5,000 rpm for 10 min using a centrifuge (Lobogen 1236R, Gyrozen Co., Daejeon, Korea). Thereafter, the supernatant was stored at -21 °C, diluted as necessary, and used in analysis experiment.

3.4 Total polyphenol content (TPC)

The TPC of OJE was measured by using a modified Folin-Denis method (Bao et al., 2021). The mixture reaction was prepared 0.14 mL of OJE to the 0.7 mL of a 0.2 N Folin-Ciocalteu reagent was reacted for 8 min at room temperature. Then the additional reaction was performed by adding 0.56 mL of 7.5% Na_2CO_3 for 60 min. The absorbance was measured at 765 nm using a spectrophotometer (Optizen 2120UV, Mecasys Co., Daejeon, Korea). The quantification of TPC was conducted by standard curve using gallic acid and the TPC of samples was expressed in mg GAE/g of DM.

3.5 Total flavonoid content (TFC)

The quantification determination TFC of OJE was measured by using a modified Kim et al. (2022), method. The 0.5 mL of OJE was prepared and mixed with 0.3 mL of 95% ethanol, 0.56 mL of DW, 0.1 mL of 1 M potassium acetate and 10% aluminum chloride which was left to reacted for 30 min at room temperature. Then, the absorbance in the react solution was measured at 415 nm using the spectrophotometer. The quantification of quercetin was used as the standard curve and the result was expressed as mg QE/g DM.

3.6 Isolation and Identification of microorganism

For microorganism screening, the solid medium was added to MRS containing 1.5% agar. Subsequently, 1.0 mL of kimchi broth diluted 100-times with MRS medium before being spread on MRS plate and cultured at 37 °C for 24 hr. Strains were harvested from agar plate, transferred into 10mL of MRS broth, and incubated for 48 hr. Following this, 18 different enzyme activities were compared using the analytical profile index (API) kit (BioMerieux Co., Lyon, France), and the strains with the highest b-glucosidase activity were selected. For the strain identification, after DNA extraction of the selected strain, the PCR was performed using 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) primers. The amplified PCR product was purified using the QIA quick PCR purification kit. The 16s rRNA gene sequence search was conducted at the NCBI using BLAST and the selected strain was called *L. plantarum* SM4.

3.7 Bioconversion

In the fermentation of *L. plantarum* SM4 used in bioconversion, the initial pH of MRS medium was adjusted to 7.0 using 1 M HCl and 1 M NaOH. The prepared MRS medium was divided to the 250 mL Erlenmeyer flask and sterilized at 121.0 °C for 15 min in an autoclave (SAC05060P, Daihan Sci., Gangwon-do, Korea). After inoculating 1.0% of *L. plantarum* SM4 in sterilized MRS medium, it was incubated at 37.0 °C and 200 rpm for 24 hr in the shaking incubator, and for bioconversion, the OJE and the fermentation medium were mixed at a ratio of 2:1 (v/v) and further cultured at 37.0 °C and 200 rpm for 48 hr. After disruption of cells with a homogenizer (HG-15A, Daihan Sci., Gangwon-do, Korea), for obtaining intracellular enzymes. After the pH of the fermentation medium was adjusted to pH 5.0 using 1 M HCl and 1 M NaOH, and then bioconversion by intracellular enzymes was induced at 45.0 °C and 200 rpm for 48 hr. The BOE was centrifuged at 4.0 °C at 5,000 rpm for 10 min, and the supernatant was separated for the analysis experiment.

3.8 HPLC-MS/MS

To confirm a qualitative analysis for determining the main component based on the molecular weight distribution in bioconversion OJE, in the Korea Institute of Basic Science (Seoul, Korea) performed with HPLC-MS/MS equipped with a ROC C18 column (3.0 × 150 mm; Restek, Saunderton, England). The OJE and BOE were filtered with a 0.22 μm PVDF syringe filter after centrifuge (M15R, Hanil Sci. Industry Co., Gimpo, Korea) by for analysis after filtration. The mobile phase was composed of solvent A (1.0% v/v formic acid/DW) and solvent B (1.0% v/v formic acid/acetonitrile) and the system gradient was operated with the following gradient composition (0 min, 95% A/5% B; 11 min, 0% A/100% B; 14 min, 0% A/100% B; 15 min, 0% A/100% B; 20 min, 95% A/5% B). N_2 gas (99.9%) was used as a spray gas for ionization, and the temperature was set to 270°C and the ion spray voltage was set to 3,000 V. The analysis results of BOE were analyzed by comparing molecular ion and fragment patterns with data library the national instrument of standard and technology (NIST) data library.

4 Conclusions

To establish the optimal UAE conditions for enhanced polyphenol extraction from OJE, extraction time, temperature, and ethanol concentration were set as independent variables and the optimal conditions were predicted by overlaying the individual response surfaces of TPC and TFC. The optimal conditions determined were extraction time of 26.0 min, extraction temperature of 92.8 °C, and 59.9% ethanol and the TPC and TFC under these conditions were found to be 5.14 ± 0.31 mg GAE/g DM and 0.59 ± 0.08 mg QE/g DM, respectively. To increase the bioactive compounds extracted from OJE, a bioconversion process using *L. plantarum* SM4 isolated from kimchi was applied. Using HPLC and HPLC-MS/MS analysis, chlorogenic acid, the main compound in OJE, was confirmed to have been converted into caffeic acid, and the content of *p*-coumaric acid was found to increase, indicating that bioconversion using *L. plantarum* SM4 was effective. Therefore, bioconversion can help increase the production of low-molecular weight polyphenols and enhance the overall bioactivity by producing useful substances through the metabolic process of *L. plantarum* SM4. Therefore, BOE, with its increased antioxidant functionality, is expected to be used as a raw material for functional foods and medicines that have a high demand for natural antioxidants. In this way, BOE

can be used as a high-value-addition raw material that is readily available and safe for human consumption.

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