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# Optimization of ultrasound-assisted extraction conditions for extraction of bioactive compounds from purple carrot (*Daucus carota L.*) using response surface methodology

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**Abstract**

Statically-based optimization was employed to maximize the total phenol content, total flavonoid content, and radical scavenging activity from purple carrot (*Daucus carota L.*) using ultrasound-assisted extraction. To enhance the extraction of bioactive components, key variables of ultrasound-assisted extraction including extraction time (5.0 ~ 55.0 min), extraction temperature (26.0 ~ 94.5 °C), and ethanol concentration (0.0 ~ 99.5%) were chosen as independent variables and optimized using Response surface methodology. The optimal extraction conditions were predicted as 41.9 °C, 46.1 min, and 26.0% ethanol, under these conditions, total phenol content, total flavonoid content, and radical scavenging activity were found to be 16.1 mg GAE/g DM, 0.86 QE/g DM, and 25.6%, respectively, similar to the experimental values of the validation experiment. When the purple carrot extract was analyzed using HPLC, chlorogenic acid was identified as a main substance. Therefore, ultrasound-assisted extraction can effectively increase the anti-oxidant production of purple carrot, and purple carrot extract has high potential as functional food and cosmetic raw material.

**Keywords:** purple carrot; optimization; ultrasound-assisted extraction; central composite design; anti-oxidant.

**Practical Application:** The extraction process using ultrasound waves was optimized to maximize the antioxidant ingredients in purple carrots, which led to the conclusion that the extract had high potential as a functional food and cosmetics raw material.

## 1 Introduction

ROS (reactive oxygen species) are formed when some oxygen during the respiratory process of aerobic organisms is converted to highly unstable oxygen by chemical, physical, and environmental stress (Madkour, 2019). They are generated *in vivo* mostly through oxidative phosphorylation and inflammatory reactions in mitochondria (Chen et al., 2019). When free radicals are produced excessively, the body performs a defense system based on anti-oxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) (Noor et al., 2022). While the rate of ROS generation is faster than the rate of scavenging, the imbalance between the generation, neutralization, and removal of ROS with the corresponding triggers in a cell signaling pathways can result in oxidative and reductive stresses with the consequent initiation of numerous pathologies, protein degradation, lipid peroxidation, and DNA mutation occur, leading to the destruction of cellular homeostasis and a rapid increase in peroxides and resulting even in cell and organismal death (Zorov et al., 2014). In addition, excessive ROS development promotes collagen oxidation, accelerates skin aging, and causes diseases such as hyperlipidemia, arteriosclerosis, cataracts, diabetes, and cancer, so the development of anti-oxidants is necessary to effectively remove ROS.

Synthetic anti-oxidants such as butylated hydroxy anisol (BHA), butylated hydroxy toluene (BHT), propylene glycol (PG), and tertiary butylhydroquinone (TBHQ), which have been developed and widely used, have adverse effects such as liver toxicity, kidney toxicity, and carcinogenesis, despite their outstanding anti-oxidant effect (Gam et al., 2020). Accordingly, the need and demand for the development of anti-oxidants derived from natural substances are increasing, which can substitute the existing synthetic anti-oxidants. Polyphenols are natural substances present in plants and are known to exhibit antioxidant ability by hydroxyl groups of aromatic compounds (Nagarajan et al., 2020). Flavonoid, which are a type of polyphenol, are glycoside compounds consisting of two aromatic rings (A and B-ring) and heterocyclic rings (C-ring) (Latos-Brozio & Masek, 2019). B-ring and C-ring of flavonoids have *ortho*-hydroxylation and C2-C3 double bonds, respectively, so they can effectively remove ROS and exhibit anti-cancer, anti-bacterial and anti-inflammatory effects (Gu et al., 2019).

Purple carrots are root vegetables in the Apiaceae family, and contain a high level of anthocyanins, a type of flavonoid (Blando et al., 2021). According to previous studies, Purple carrots are known to be richer in polyphenols and vitamin C than orange carrots, and have been reported to have about three times greater antioxidant activity (Nicolle et al., 2004). In particular, it is known that β-carotene and anthocyanin prevent cardiovascular diseases and diabetes and have anti-cancer effects, thus there is

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an increasing interest in the functionality of bioactive substances that purple carrots contain (Rasheed et al., 2022). However, studies on the optimization of extraction conditions to increase extraction of bioactive substances in Purple carrot extract (PCE) and standardization of the process are lacking.

Therefore, in this study, extraction time, extraction temperature, and the ethanol concentration were set as independent variables, using the environment-friendly process, ultrasonic extraction, to effectively extract biologically active substances from purple carrots, and for more effective optimization of extraction conditions, the response surface methodology (RSM), a type of statistical optimization, was applied to optimize the main process variables. Through this, our study proposed an eco-friendly extraction process that can increase the extraction efficiency of anti-oxidants from purple carrots, thereby increasing the extraction of high value-added substances from purple carrots.

## 2 Materials and methods

### *2.1 Raw materials and reagents*

The purple carrot dried powder used in this study was purchased from Purunsan (Seoul, Korea), agricultural corporation. 2 N Folin-ciocalteu, potassium acetate, aluminum chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), citric acid, gallic acid, quercetin, ascorbic acid, acetonitrile, and acetic acid required for the measurement of anti-oxidant activity and HPLC analysis were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Sodium carbonate for the measurement of anti-oxidant activity was purchased from Duksan Pharmaceutical Co., Ltd. (Ansan, Korea) and all other reagents used for the analysis were Sigma-Aldrich's extra pure reagents.

### *2.2 Ultrasound-assisted extraction (UAE)*

UAE was used to extract physiologically active substances, and 1.0 g of purple carrot powder and 20.0 mL ethanol for each concentration were mixed in a pressure vessel, and an ultrasonicator (SD-250H, Mujigae Co., Seoul, Korea) was used for the extraction. A mixture of distilled water and 99.5% ethanol (Samchun, Seoul, Korea) was used as the extraction solvent, and extraction was performed by varying the extraction time and extraction temperature. After centrifuging the extract at 5,000 rpm for 10.0 min for solid-liquid separation, the supernatant was harvested, refrigerated, and used for analysis.

### *2.3 Total polyphenol content (TPC)*

TPC was used with modifications of Dewanto's method based on the principle that Folin-ciocalteu is reduced by polyphenol and turns blue (Ceccanti et al., 2022). After mixing 0.7 mL of 0.2 N Folin-ciocalteu's phenol reagent with 0.14 mL of PCE, and reacting for 8.0 min, 0.56 mL of 7.5% $Na_2CO_3$ was added and reacted for 60.0 min. The absorbance of the mixture was measured at 765 nm in spectrophotometer (Optizen 2120 UV, Mecasys Co., Daejeon, Korea), and the TPC was expressed as mg gallic acid equivalent (GAE)/g dry matter (DM) using standard calibration curve plotted by gallic acid.

### *2.4 Total flavonoid content (TFC)*

TFC was measured by modifying Mussatto's method (Ozuna et al.,2020). 0.1 mL of PCE, 0.56 mL of distilled water and 0.3 mL of 99.5% ethanol were added, and 1 M potassium acetate and 10.0% aluminum chloride were sequentially added by 0.02 mL each for a color development. The mixture reacted for 30.0 min to induce color development, and absorbance was measured at 415 nm in a spectrometer. TFC was quantified as a relative concentration with mg quercetin equivalent (QE)/g DM through a standard calibration curve using quercetin.

### *2.5 Radical scavenging activity (RSA)*

RSA was measured based on modified Brand-Williams' method (Akbar et al., 2022). After adjusting the absorbance of the DPPH solution diluted with methanol at 1.0 (± 0.02), 1.25 mL of 0.1 mM DPPH was added to 0.25 mL of PCE. After the mixture was allowed to react in the dark for 20.0 min, absorbance was measured at 517 nm. For the quantification of RSA, ascorbic acid was used as a positive control to calculate radical scavenging activity by the following Formula 1.

$$\mathrm{RSA}(\%)=\left(1-\frac{\text{absorbance of extract – added group}}{\text{absorbance of control group}}\right)\times 100 \quad (1)$$

### *2.6 Statistically-based optimization*

In order to maximize the production of bioactive substances, RSM based central composite design (CCD), Design Expert 9.0 (Stat-Ease Inc., Minneapolis, USA) was applied to generate regression models for the prediction of UAE conditions. CCD with 3 variables with five levels was used to derive 17 experimental conditions (Table 1). The independent variables ($X_n$) were used in our experiment are extraction time ($X_1$, 5.0 ~ 55.0 min), extraction temperature ($X_2$, 26.0 ~ 94.0 °C), and ethanol concentration ($X_3$, 0.0 ~ 99.5%), and the dependent variable was TPC, TFC, and RSA were used. The quadratic regression equation for the independent variable and the dependent variable was derived as follows, and only variables recognized for significance through

**Table 1**. Main independent variables and levels for the extraction of bioactive compound from purple carrot based on coded and actual values of central composite design.

| $X_n$ | Independent variables | Coded levels | | | | |
|---|---|---|---|---|---|---|
| | | -1.68 | -0.84 | 0 | +0.84 | +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 | 94.5 |
| $X_3$ | Ethanol concentration (%) | 0.0 | 20.0 | 50.0 | 80.0 | 99.5 |

$X_n$: independent variables, $X_1$: extraction time (min), $X_2$: extraction temperature (°C), $X_3$: ethanol concentration (%).

ANOVA and regression analysis were adopted. In addition, the interaction between the univariate optimization curve and the three-dimensional response surface curve and the variables was confirmed, and the response surface of the dependent variable according to the change of the independent variable was analyzed using Formula 2.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_1^2X_1X_2 + b_1^3X_1X_3 + b_2^3X_2X_3 + b_1^1X_1^2 + b_2^2X_2^2 + b_3^3X_3^2 \quad (2)$$

Y is process response, $X_n$ is the coded independent variables, $X_n^2$ is the quadratic term of the independent variable, $b_0$ is the y-intercept, and $b_n$ is the regression coefficient.

### *2.7 Measurment of main substances*

To detect main substances contained in purple carrots, the PCE was filtered with a 0.22 μm syringe filter (PVDF2025A, Hyundai Micro Co., Seoul, Korea), and HPLC analysis was performed using a Poroshell 120 EC-C18 column (4.6 mm × 150 mm, Agilent Tech., Santa Clara, California, USA). Mobile phases were used by changing the mixing ratio of mobile phase A (99.9% acetonitrile) and mobile phase B (1.0% acetic acid) according to the separation time. For identification of components in extract, the flow rate was fixed at 0.5 mL/min for the comparison of retention time, and the comparison of absorption spectrum was applied to reconfirm the compounds identified based on retention time at 190 ~ 640 nm using diode array detector (DAD). The detection time and absorption spectrum were compared with the standard material and a calibration curve based on the peak area was used for the quantification.

### *2.8 Statistical analysis*

All experiments conducted for functional evaluation of purple carrots based on optimal extraction conditions were repeated three times and expressed as mean ± standard deviation. Mean values, standard deviations and statistical numerical calculations were performed using GraphPad Prism (Ver. 5.0, GraphPad software Inc., California, USA).

## 3 Results

### *3.1 Exploring TPC optimal extraction conditions*

Compared with experimental values depending on each of the 17 conditions, the maximum value of TPC was measured 18.0 mg GAE/g DM at Run #4, while the minimum value was measured to be 6.5 mg GAE/g DM at Run #14 (Table 2). The correlation between the independent variable and the dependent variable according to the experimental conditions is shown in the quadratic regression equation and the coefficient of determination in Table 3. The *p*-value is the probability that

**Table 2**. Central composite design matrix for independent variables with experimental values of Total polyphenol content, Total flavonoid content, and Radical scavenging activity.

| Run No. | Extraction conditions | | | TPC (mg GAE/g DM) | TFC (mg QE/g DM) | RSA (%) |
|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ | | | |
| 1 | 15.0 | 40.0 | 20.0 | 15.8 | 0.40 | 42.6 |
| 2 | 45.0 | 40.0 | 20.0 | 15.8 | 0.39 | 42.2 |
| 3 | 15.0 | 80.0 | 20.0 | 16.2 | 0.48 | 59.5 |
| 4 | 45.0 | 80.0 | 20.0 | 18.0 | 0.35 | 51.8 |
| 5 | 15.0 | 40.0 | 80.0 | 12.3 | 0.57 | 40.0 |
| 6 | 45.0 | 40.0 | 80.0 | 12.0 | 0.60 | 46.6 |
| 7 | 15.0 | 80.0 | 80.0 | 14.3 | 0.68 | 47.2 |
| 8 | 45.0 | 80.0 | 80.0 | 14.0 | 0.66 | 56.2 |
| 9 | 5.0 | 50.0 | 50.0 | 16.3 | 0.41 | 71.6 |
| 10 | 55.0 | 50.0 | 50.0 | 17.3 | 0.40 | 72.7 |
| 11 | 30.0 | 50.0 | 50.0 | 16.6 | 0.39 | 62.9 |
| 12 | 30.0 | 94.0 | 50.0 | 17.2 | 0.53 | 84.7 |
| 13 | 30.0 | 60.0 | 0.0 | 15.9 | 0.19 | 28.7 |
| 14 | 30.0 | 60.0 | 99.5 | 6.5 | 0.62 | 23.2 |
| 15 | 30.0 | 60.0 | 50.0 | 16.9 | 0.53 | 70.4 |
| 16 | 30.0 | 60.0 | 50.0 | 17.1 | 0.53 | 71.8 |
| 17 | 30.0 | 60.0 | 50.0 | 17.4 | 0.56 | 72.7 |

$X_1$: extraction time (min), $X_2$: extraction temperature (°C), $X_3$: ethanol concentration (%), TPC: total phenolic content, TFC: total flavonoid content, RSA: radical scavenging activity.

**Table 3**. Second-order polynomial equations generated by central composite design for the optimization of extraction conditions of purple carrot.

| Responses | Regression models | $R^2$ | *p* |
|---|---|---|---|
| TPC (mg GAE/g DM) | $Y_{TPC} = +12.54946 + 0.037584X_1 + 0.015369X_2 + 0.17187X_3 + 7.62917X_1X_2 - 7.12056X_1X_3 + 2.9312X_2X_3 - 5.66267X_1^2 - 2.07775X_2^2 - 2.39853X_3^2$ | 0.9506 | 0.0009 |
| TFC (mg QE/g DM) | $Y_{TFC} = +10.38222 + 0.24887X_1 + 0.74118X_2 + 1.92733X_3 - 2.0416X_1X_2 - 6.58333X_1X_3 - 2.02083X_2X_3 - 6.83164X_1^2 - 2.38439X_2^2 - 0.020451X_3^2$ | 0.8706 | 0.0201 |
| RSA (%) | $Y_{RSA} = +0.011382 + 9.19603X_1 + 4.89458X_2 + 4.46105X_3 - 7.08333X_1X_2 + 4.22222X_1X_3 + 2.62500X_2X_3 - 1.30352X_1^2 - 2.07318X_2^2 - 3.35553X_3^2$ | 0.9558 | 0.006 |

$R^2$: coefficient of determination, $X_1$: extraction time (min), $X_2$: extraction temperature (°C), $X_3$: ethanol concentration (%), $Y_{TPC}$: predicted response of TPC, $Y_{TFC}$: predicted response of TFC, $Y_{RSA}$: predicted response of RSA.

more events against the null hypothesis occur than events observed under the null hypothesis, and the smaller the *p*-value, the more statistically significant it can be interpreted (Kim & Park 2019; Gam et al., 2021). Rejection is decided based on the significance probability of 0.05. TPC was confirmed as $p < 0.0009$, which was smaller than 0.05, so it was possible to reject the null hypothesis and confirm that it was statistically significant. Also, the concentration of ethanol was found to be $p < 0.0001$ in the linear and quadratic terms, indicating that the ethanol concentration is a major variable that has a significant effect on TPC.

As the ethanol concentration increased, TPC increased up to the maximum value of 17.7 mg GAE/g DM and then decreased, verifying that the ethanol concentration had a significant effect on the TPC variance (Figure 1). The interactions between two independent variables on TPC was presented using visualization through 3D response surface plot (Figure 2). In both Figure 2B and Figure 2C, it was confirmed that the ethanol concentration had a large effect on the TPC variation, whereas the extraction temperature and extraction time did not have a significant effect. Based on RSM, the optimized TPC extraction conditions for purple carrots were predicted to be 32.5 min, 76.3 °C, and 38.0% ethanol, under these, TPC was derived to be maximum of 18.1 mg GAE/g DM. This result was consistent with the results of Lee (2016), in which the ethanol concentration was proportional to TPC at less than 20.0% and the ethanol concentration was inversely proportional at 50.0% or more. It is known to use a mixed solvent of ethanol and water rather than a single solvent to increase the solubility of polyphenols in the extraction solvent when extracting plant polyphenols (Lohvina et al., 2021). According to the study results that polyphenol contained in natural substances have multipolarity depending on hydroxyl groups, molecular weight, and length of hydrocarbons, and that the polarity changes of a binary solvent of water and ethanol affects the extraction yield (Kong et al., 2021). Therefore, it is considered that the optimization of the ethanol concentration should be prioritized for optimal PCE.

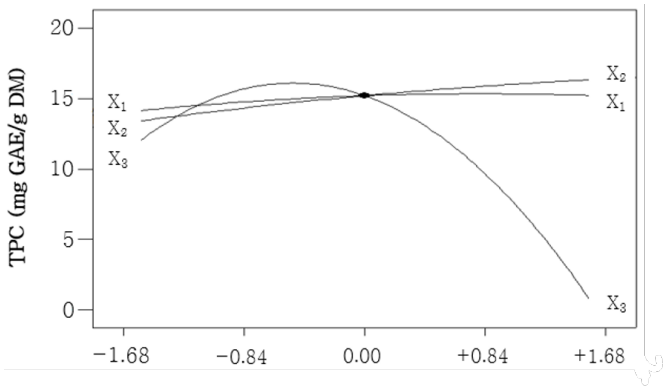


**Figure 1**. Univariate optimization graph of TPC affected by each independent variable, including extraction time ($X_1$), extraction temperature ($X_2$), and ethanol concentration ($X_3$).

### *3.2 Exploring TFC optimal extraction conditions*

This experiment was intended to maximize TFC, and the changes in the experimental values depending on the conditions shown in Table 2 were observed. The maximum value of TFC was found to be 0.68 mg QE/g DM at Run #7, while the minimum value was measured to be 0.19 mg QE/g DM at Run #13. $R^2$ of the quadratic regression equation, was 0.9113, and the effectiveness of prediction and the significance of the regression model were verified with $p < 0.006$ (Table 3).

Contrary the previous TPC results, TFC increased as ethanol concentration decreased, and a maximum value was 0.7 mg QE/g DM (Figure 3). In Figure 4A, the TFC for the extraction temperature showed the maximum value at 0.5 mg QE/g DM, and it can be seen that the TFC decreased as the extraction time increased, and through this, the correlation between the extraction time and the extraction temperature ($X_2$, $X_3$: $p < 0.0135$), which was consistent with the study of Lee et al. (2018).

In Figure 4B and 4C, TFC decreased with increasing ethanol concentration, and the maximum values were found to be 0.40 mg QE/g DM and 0.5 mg QE/g DM, respectively. The optimized TFC extraction conditions for purple carrots were predicted to be 33.4 min in terms of extraction time, with extraction temperature of 49.2 °C, and 3.0% concentration of ethanol, deriving TFC of 0.9 mg QE/g DM. Using mixed solvents, the normalized polarity increased as the concentration of ethanol decreased (Huaman-Castilla et al., 2019). Accordingly, it is expected that TFC increased as the solubility of flavonoid with high polarity increased due to an increase in the polarity of the extraction solvent under the condition of 3.0% ethanol concentration. Particularly, anthocyanins, which are highly contained in purple carrots, exist as glycoside combined with

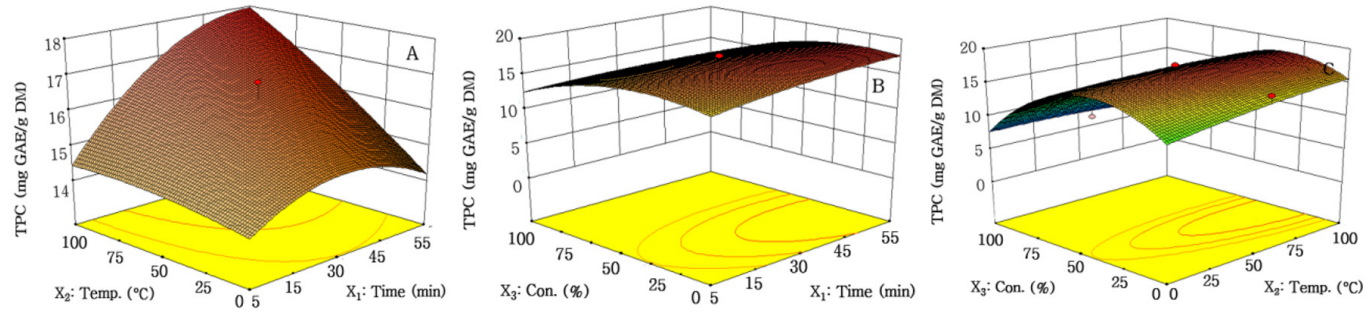


**Figure 2.** 3D response surface graph for the evaluation of interactive effects of independent variables on TPC of the PCE.

cyanidin (Corinti et al., 2019). As a result of comparing the antioxidant activity of honeyberry extracts by Lee et al. (2016), the lower the concentration of ethanol, the higher the anthocyanin content. Based on the research results of kim that the higher the ratio of water in the water and ethanol mixed solvent, the more the tissue of the sample swells, and the penetration into the inside of the sample is easy (Kim, 2016). Therefore, UAE using 3.0% ethanol liberates anthocyanins and is considered to have an effect of improving biological activity.

### *3.3 Exploring RSA optimal extraction conditions*

This experiment was intended to maximize RSA, and the changes in the experimental values depending on the conditions shown in Table 2 were observed. The maximum value of RSA was measured to be 84.7% at Run #12, and the minimum value was measured to be 23.2% at Run # 14, indicating that RSA was affected by extraction temperature and the ethanol concentration.

The coefficient of determination (R-squared) $R^2$ has a value between 0.0 and 1.0 and the closer to 1.0, higher suitability of the quadratic regression equation is judged (Yeom et al., 2022). The $R^2$ of the second regression equation was 0.9558, showing suitability of 95.0% or higher, and the validity of the optimal condition prediction using the second regression equation was recognized (Table 3). Depending on changes in independent variables, RSA was explored through a univariate optimization graph, but the variation range of RSA was not big depending on extraction time and extraction temperature (Figure 5).

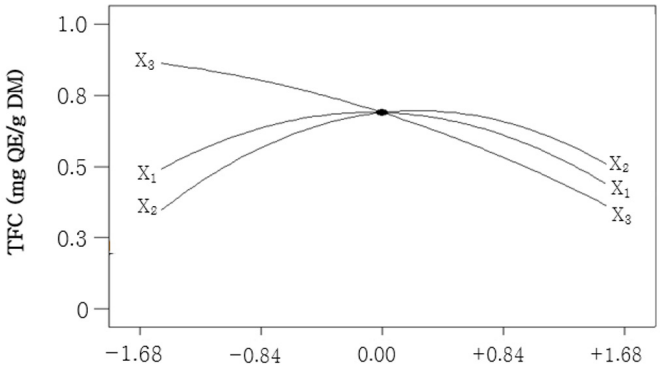


**Figure 3**. Univariate optimization graph of TFC affected by each independent variable, including extraction time ($X_1$), extraction temperature ($X_2$), and ethanol concentration ($X_3$).

However, in the case of ethanol concentration, the graph showed the maximum value at 84.7%. In addition, interactions between independent variables were visualized using 3D response surface graph (Figure 6). Consequently, there was no significant change in the extraction time, it was found through a graph in which RSA increased according to the extraction temperature confirmed (Figure 6A). In Figure 6B and 6C, RSA according to the change in ethanol concentration showed maximum values at 69.4% and 79.8%, respectively. As extraction temperature increased, RSA increased, whereas it was found that the response variation range of extraction time was not wide. Based on the previous results, the optimized extraction conditions for purple carrots were derived as an extraction temperature of 94.0 °C, ethanol concentration of 47.0%, and extraction time of 27.0 min and RSA was predicted to be 79.6%. Dorosh et al. reported that the proportional relationship between the extraction temperature and RSA was due to the new formation of low molecular polyphenols, and Kaur et al. reported that the mixed solvent of ethanol and water affected RSA (Dorosh et al., 2020; Kaur et al., 2022). Therefore, it is presumed that this result is because the newly formed phenolic hydroxyl groups of purple carrots extracted by the mixed solvent do not form a pair and thus have reducing power to eliminate or stabilize radicals by providing electrons to highly reactive radicals (Ahn et al., 2015). Therefore, it is considered that extraction temperature and ethanol concentration should be prioritized for maximizing purple carrot RSA.

### *3.4 Exploring the optimal extraction conditions*

To establish the optimal extraction conditions, the common optimized extraction conditions were predicted by overlaying the individual response surface curves of TPC, TFC, and RSA, which were predicted using the quadratic regression equation. In the scope of the optimal extraction conditions including the extraction time for 5.0 ~ 55.0 min, the extraction temperatures at 26.0 ~ 94.0°C, and 0.0 ~ 99.5% ethanol, the concentration of ethanol which was confirmed to be a major variable, was preferentially optimized. Considering economic feasibility, the minimization conditions of extraction time and extraction temperature were selected as limiting conditions. This derived the optimal extraction conditions including the extraction time for 46.1 min, the extraction temperature at 41.9 °C, and 26.0% ethanol, where TPC, TFC, and RSA were predicted to be 15.86 mg GAE/g DM, 0.91 mg QE/g DM, and 24.96%, respectively (Figure 7). This was similar to the experimental values in the verification experiment, thus reliability of the derived quadratic

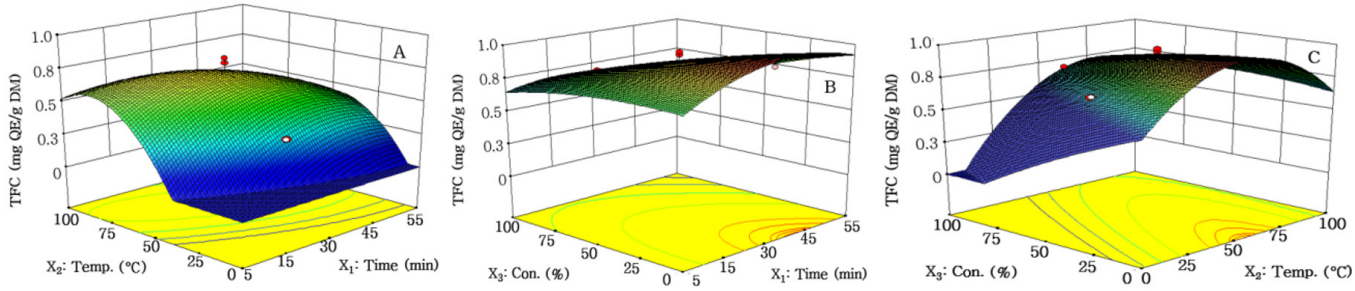


**Figure 4.** 3D response surface graph for the evaluation of interactive effects of independent variables on TFC of the PCE.

regression equation and the optimal extraction conditions in this experiment were reconfirmed to be suitable for optimization.

### *3.5 Main substances*

To explore the main substances contained in PCE, purple carrots were extracted using the conditions derived through statistical optimization, and the main substances were quantitatively analyzed through HPLC (high-performance liquid chromatography). The main peak of the optimal PCE was 11.52 min, and peak of chlorogenic acid, which is one of reference materials, was 11.52 min, confirming that the retention time was the same (Figure 8). In a comparison of absorption spectrum, the absorbance pattern and maximum absorbance showed the same pattern, confirming that the main material was chlorogenic acid. Using the peak area, the concentration of chlorogenic acid was calculated, showing the result of 0.47 mg/g.

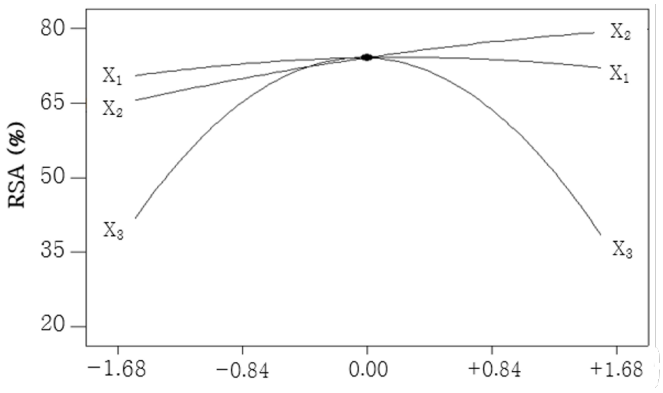


**Figure 5**. Univariate optimization graph of RSA affected by each independent variable, including extraction time ($X_1$), extraction temperature ($X_2$), and ethanol concentration ($X_3$).

Chlorogenic acid, which is a type of polyphenol, belongs to cinnamic acids, and is a compound where a hydroxyl group and a carboxyl group (-COOH) are ester-bonded, and its various biological activities are being reported. Recently, chlorogenic acid, which has the highest content among physiologically active substances in burdock root extract, inhibits advanced glycation end products (AGEs), which cause diseases such as diabetes complications, aging, and arteriosclerosis, which are produced due to chemical stress Research results have been reported (Kim et al., 2011). Therefore, it is deemed that based on the efficacy of chlorogenic acid in the optimized extract of purple carrots, its usefulness as various functional foods will be increased.

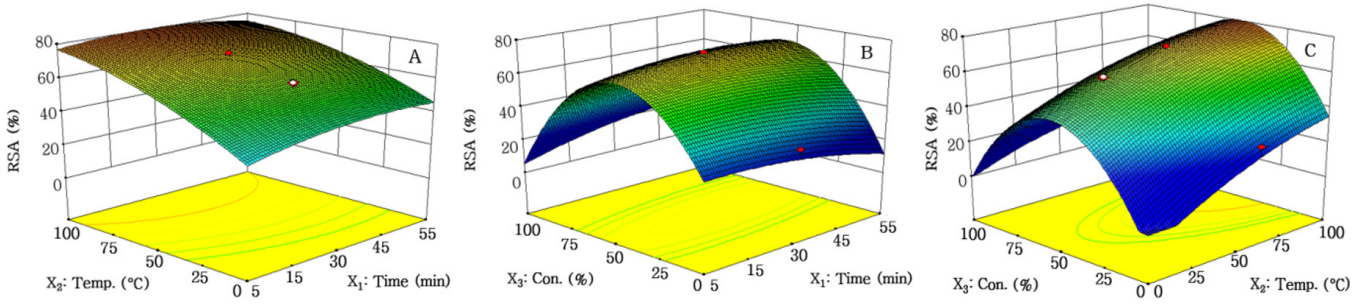


**Figure 6.** 3D response surface graph for the evaluation of interactive effects of independent variables on RSA of the PCE.

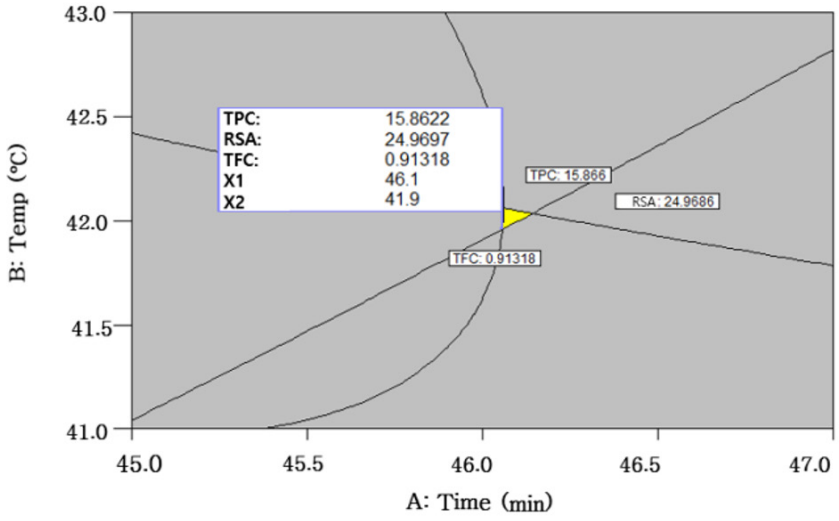


**Figure 7.** Superimposed plots showing the optimized condition of extraction time, extraction temperature, and ethanol concentration.

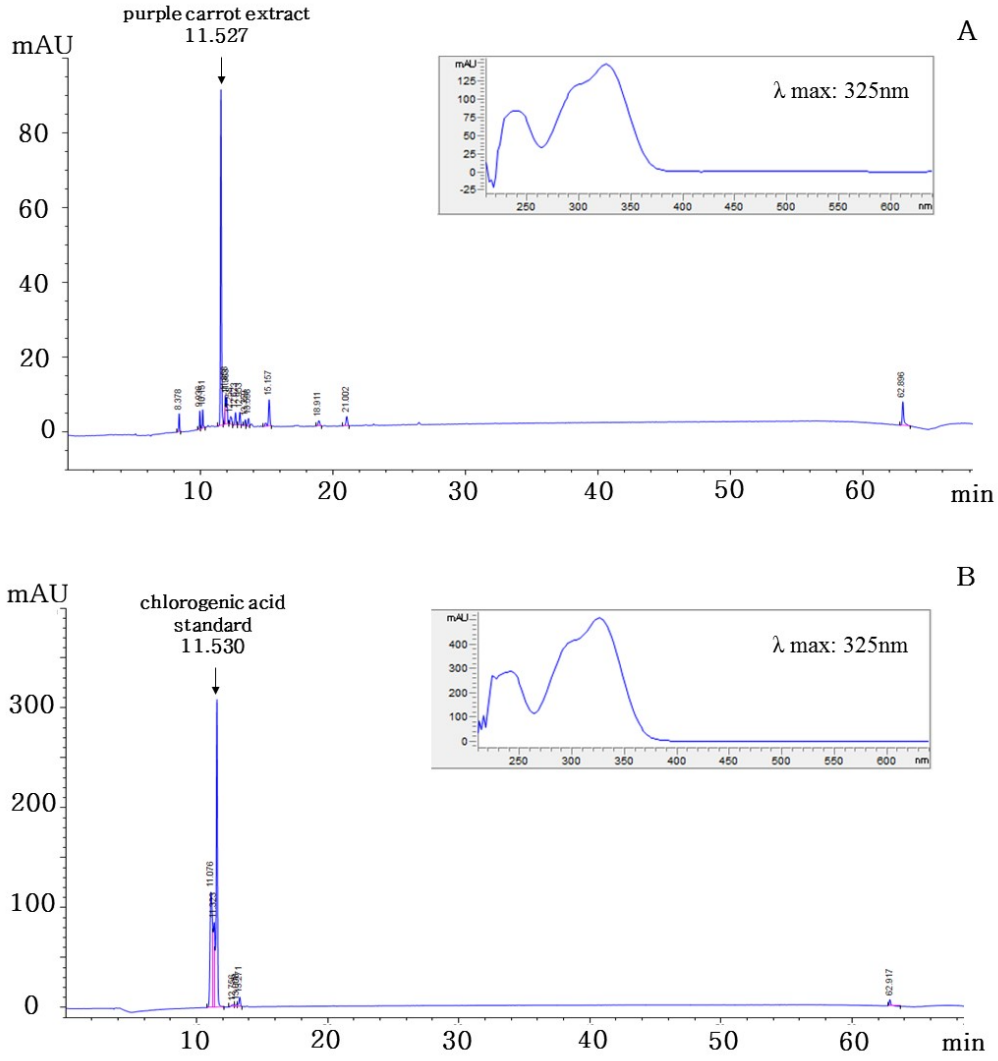


**Figure 8.** HPLC analysis to identify the main components of PCE. A: PCE, B: chlorogenic acid standard.

## 4 Conclusions

In this study, conditions for the ultrasonic extraction process were optimized using CCD to effectively extract biologically active substances in purple carrots. The optimal extraction condition to maximize dependent variables including TPC, TFC, and RSA, were predicted to extraction temperature of 41.9 °C, extraction time of 46.1 min, and ethanol 26.0%, under these conditions. To verify this, TPC, TFC, and RSA were performed, with results of 15.86 mg GAE/g DM in TPC, 0.91 mg QE/g DM in TFC, and 24.96% RSA, showing the similar values to the predicted values. Thus, it was found that the quadratic regression equation was reliable. The PCE was analyzed using HPLC, and the results showed that chlorogenic acid was the main substance. From the above results, purple carrots are expected to enhance high value-added revenue as a functional ingredient for biological activities. It is considered that the effects of preventing and delaying aging and degenerative diseases can be expected by reducing oxidative damage in the human body as purple carrots exhibited a high possibility to be a medicinal ingredient in the future.

## References

Ahn, H. Y., Choe, D. J., & Cho, Y. S. (2015). Antioxidant activity and chemical characteristics of orostachys malacophyllus and fermented orostachys Malacophyllus. *Journal of Life Science*, 25(5), 577-584. http://dx.doi.org/10.5352/JLS.2015.25.5.577.

Akbar, A., Soekamto, N. H., Firdaus, & Bahrun. (2022). Total phenolics and flavonoids level of n-hexane, ethyl acetate and methanol extracts of *Sargassum* sp. along with their antioxidant activity by DPPH method. *AIP Conference Proceedings*, 2638, 060009. https://doi.org/10.1063/5.0104885.

Blando, F., Marchello, S., Maiorano, G., Durante, M., Signore, A., Laus, M. N., Soccio, M., & Mita, G. (2021). Bioactive compounds and antioxidant capacity in anthocyanin-rich carrots: a comparison between the black carrot and the apulian landrace 'Polignano' Carrot. *Plants*, 10(3), 564. http://dx.doi.org/10.3390/plants10030564. PMid:33802658.

Ceccanti, C., De Bellis, L., Guidi, L., Negro, C., Pardossi, A., & Incrocci, L. (2022). Effect of blanching and boiling on the secondary metabolism of cultivated cardoon stalks: a case study of the tuscany region (Italy).

*Metabolites*, 12(8), 728. http://dx.doi.org/10.3390/metabo12080728. PMid:36005600.

Chen, Y., Yang, M., Huang, W., Chen, W., Zhao, Y., Schulte, M. L., Volberding, P., Gerbec, Z., Zimmermann, M. T., Zeighami, A., Demos, W., Zhang, J., Knaack, D. A., Smith, B. C., Cui, W., Malarkannan, S., Sodhi, K., Shapiro, J. I., Xie, Z., Sahoo, D., & Silverstein, R. L. (2019). Mitochondrial metabolic reprogramming by CD36 signaling drives macrophage inflammatory responses. *Circulation Research*, 125(12), 1087-1102. http://dx.doi.org/10.1161/CIRCRESAHA.119.315833. PMid:31625810.

Corinti, D., Maccelli, A., Crestoni, M. E., Cesa, S., Quaglio, D., Botta, B., Ingallina, C., Mannina, L., Tintaru, A., Chiavarino, B., & Fornarini, S. (2019). IR ion spectroscopy in a combined approach with MS/MS and IM-MS to discriminate epimeric anthocyanin glycosides (Cyanidin 3-O-Glucoside and-Galactoside). *International Journal of Mass Spectrometry*, 444, 116179. http://dx.doi.org/10.1016/j.ijms.2019.116179.

Dorosh, O., Moreira, M. M., Pinto, D., F Peixoto, A., Freire, C., Costa, P., Rodrigues, F., & Delerue-Matos, C. (2020). Evaluation of the extraction temperature influence on polyphenolic profiles of vine-canes (*Vitis Vinifera*) subcritical water extracts. *Foods*, 9(7), 872. http://dx.doi.org/10.3390/foods9070872. PMid:32635200.

Gam, D. H., Park, J. H., Hong, J. W., Jeon, S. J., Kim, J. H., & Kim, J. W. (2021). Effects of *Sargassum thunbergii* extract on skin whitening and anti-wrinkling through inhibition of TRP-1 and MMPs. *Molecules (Basel, Switzerland)*, 26(23), 7381. http://dx.doi.org/10.3390/molecules26237381. PMid:34885963.

Gam, D. H., Yi Kim, S., & Kim, J. W. (2020). Optimization of ultrasound-assisted extraction condition for phenolic compounds, antioxidant activity, and epigallocatechin gallate in lipid-extracted microalgae. *Molecules (Basel, Switzerland)*, 25(3), 454. http://dx.doi.org/10.3390/molecules25030454. PMid:31973236.

Gu, Q., Duan, G., & Yu, X. (2019). Bioconversion of flavonoid glycosides from Hippophae rhamnoides leaves into flavonoid aglycones by Eurotium amstelodami. *Microorganisms*, 7(5), 122. http://dx.doi.org/10.3390/microorganisms7050122. PMid:31060344.

Huaman-Castilla, N. L., Martínez-Cifuentes, M., Camilo, C., Pedreschi, F., Mariotti-Celis, M., & Pérez-Correa, J. R. (2019). The impact of temperature and ethanol concentration on the global recovery of specific polyphenols in an integrated HPLE/RP process on Carménère Pomace extracts. *Molecules (Basel, Switzerland)*, 24(17), 3145. http://dx.doi.org/10.3390/molecules24173145. PMid:31470596.

Kaur, R., Manchanda, P., & Sidhu, G. S. (2022). Optimization of extraction of bioactive phenolics and their antioxidant potential from callus and leaf extracts of stevia rebaudiana bertoni. *Journal of Food Measurement and Characterization*, 16(1), 461-470. http://dx.doi.org/10.1007/s11694-021-01176-2.

Kim, J., Jeong, I. H., Kim, C. S., Lee, Y. M., Kim, J. M., & Kim, J. S. (2011). Chlorogenic acid inhibits the formation of advanced glycation end products and associated protein cross-linking. *Archives of Pharmacal Research*, 34(3), 495-500. http://dx.doi.org/10.1007/s12272-011-0319-5. PMid:21547683.

Kim, M. K. (2016). Optimal extraction conditions of active components from the adventitious roots of noni (Morinda Citrifolia). *Korean Journal of Food Science Technology*, 48(2), 111-116. http://dx.doi.org/10.9721/KJFST.2016.48.2.111.

Kim, T. K., & Park, J. H. (2019). More about the basic assumptions of T-Test: normality and sample size. *Korean Journal of Anesthesiology*, 72(4), 331-335. http://dx.doi.org/10.4097/kja.d.18.00292. PMid:30929413.

Kong, F., An, Y., Jiang, L., Tian, J., Yang, M., Li, M., Zhang, Z., Guan, B., Zheng, Y., & Yue, X. (2021). Spectroscopic and docking studies of the interaction mechanisms of xylitol with α-Casein and κ-Casein. *Colloids and Surfaces. B, Biointerfaces*, 206, 111930. http://dx.doi.org/10.1016/j.colsurfb.2021.111930. PMid:34182429.

Latos-Brozio, M., & Masek, A. (2019). Structure-activity relationships analysis of monomeric and polymeric polyphenols (Quercetin, Rutin and Catechin) obtained by various polymerization methods. *Chemistry & Biodiversity*, 16(12), e1900426. http://dx.doi.org/10.1002/cbdv.201900426. PMid:31657102.

Lee, G. D. (2016). Monitoring of antioxidant activities with dried gugija (Lycium Chinensis Mill) extraction. *Korean Journal of Food Preservation*, 23(6), 859-865. http://dx.doi.org/10.11002/kjfp.2016.23.6.859.

Lee, S. B., Wang, X., & Yoo, B. H. (2018). Optimization of total flavonoids extraction process from wheat sprout using central composite design model. *Applied Chemistry for Engineering*, 29(4), 446-451. https://doi.org/10.14478/ace.2018.1027.

Lee, Y. S., Yoo, J. H., & Lee, H. J. (2016). Comparative evaluation of extraction and processing methods on antioxidative contents and radical scavenging activity of honeyberry. *FoodService Industry Journal*, 12(4), 35-46. http://dx.doi.org/10.22509/kfsa.2016.12.4.003.

Lohvina, H., Sándor, M., & Wink, M. (2021). Effect of Ethanol Solvents on Total Phenolic Content and Antioxidant Properties of Seed Extracts of Fenugreek (*Trigonella Foenum-Graecum L.*) Varieties and Determination of Phenolic Composition by HPLC-ESI-MS. *Diversity (Basel)*, 14(1), 7. http://dx.doi.org/10.3390/d14010007.

Madkour, L. H. (2019). Function of Reactive Oxygen Species (ROS) inside the Living Organisms and Sources of Oxidants. *Journal of Pharmaceutical Sciences and Research*, 2, 180023.

Nagarajan, S., Nagarajan, R., Kumar, J., Salemme, A., Togna, A. R., Saso, L., & Bruno, F. (2020). Antioxidant activity of synthetic polymers of phenolic compounds. *Polymers*, 12(8), 1646. http://dx.doi.org/10.3390/polym12081646. PMid:32722059.

Nicolle, C., Simon, G., Rock, E., Amouroux, P., & Rémésy, C. (2004). Genetic variability influences carotenoid, vitamin, phenolic, and mineral content in white, yellow, purple, orange, and dark-orange carrot cultivars. *American Society for Horticultural Science*, 129(4), 523-529. http://dx.doi.org/10.21273/JASHS.129.4.0523.

Noor, K. K., Ijaz, M. U., Ehsan, N., Tahir, A., Yeni, D. K., Neamul Kabir Zihad, S. M., Uddin, S. J., Ashraf, A., & Simal-Gandara, J. (2022). Hepatoprotective role of vitexin against cadmium-induced liver damage in male rats: a biochemical, inflammatory, apoptotic and histopathological investigation. *Biomedicine and Pharmacotherapy*, 150, 112934. http://dx.doi.org/10.1016/j.biopha.2022.112934. PMid:35421786.

Ozuna, C., Mulík, S., Valdez-Rodríguez, B., Abraham-Juarez, M. D. R., & Fernández-López, C. L. (2020). The effect of organic farming on total phenols, total flavonoids, brown compounds and antioxidant activity of spent coffee grounds from Mexico. *Biological Agriculture and Horticulture*, 36(2), 107-118. http://dx.doi.org/10.1080/01448765.2019.1704876.

Rasheed, H., Shehzad, M., Rabail, R., Kowalczewski, P. Ł., Kidoń, M., Jeżowski, P., Ranjha, M. M. A. N., Rakha, A., Din, A., & Aadil, R. M. (2022). Delving into the nutraceutical benefits of purple carrot against metabolic syndrome and cancer: a review. *Applied Sciences (Basel, Switzerland)*, 12(6), 3170. http://dx.doi.org/10.3390/app12063170.

Yeom, S. H., Gam, D. H., Kim, J. H., & Kim, J. W. (2022). Development of ultrasound-assisted extraction to produce skin-whitening and anti-wrinkle substances from safflower seed. *Molecules (Basel, Switzerland)*, 27(4), 1296. http://dx.doi.org/10.3390/molecules27041296. PMid:35209083.

Zorov, D. B., Juhaszova, M., & Sollott, S. J. (2014). Mitochondrial Reactive Oxygen Species (ROS) and ROS-Induced ROS Release. *Physiological Reviews*, 94(3), 909-950. http://dx.doi.org/10.1152/physrev.00026.2013. PMid:24987008.