

Technical paper

Optimization of Hot-water Extraction of Dried Yacon Herbal Tea Leaves: Enhanced Antioxidant Activities and Total Phenolic Content by Response Surface Methodology

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Yacon (*Smallanthus sonchifolius*) is an herbal plant and its root has been historically consumed as a sweet vegetable in the Andes. With the research goal of establishing yacon leaves, grown in Japan, as a foodstuff with health benefits, we previously reported the antioxidant effects of yacon tea leaves using concentrated extracts. In this study, we determined the optimum conditions for regular hot-water extraction of yacon tea leaves, intended for normal consumption, aiming for higher activity in several antioxidant assays and total phenolic content (TPC). Response surface methodology was used to optimize the extraction by central composite design. Extraction temperature (ranging from 75.0 to 96.0 °C) and time (from 2.00 to 5.50 min) were set as the two independent variables. Based on a composite desirability value of 0.863, the hot-water extraction of yacon tea at 89.3 °C for 2.50 min was found to be the optimized condition providing higher antioxidant activity and TPC.

Keywords: yacon tea leaves, antioxidant, response surface methodology, central composite design, optimization

Introduction

Yacon (*Smallanthus sonchifolius*) is a perennial plant originally cultivated in the Andean highlands of South America. Over the past decades, yacon has been introduced to other regions such as Asia, Oceania, and Europe (Ojansivu *et al.*, 2011). The root of this plant has the shape of a sweet potato and it has been historically consumed as a sweet vegetable. It is currently also available in processed foods, such as syrup, juice, ice cream, candy, and pickled vegetables in local markets in Japan. Occasionally, yacon leaves are used as an herbal tea and consumed in Japan; however, most of the aerial part of the

plant remains to be unused after harvest of the root. Yacon leaves possess antifungal (Lin *et al.*, 2003) and antioxidant activities, and exert a hypoglycemic effect in both normal and diabetic rat models (Aybar *et al.*, 2001). Chlorogenic, caffeic, and ferulic acids have been isolated and identified as the major phenolic constituents of yacon leaves (Valentova *et al.*, 2005; 2006). The phenolic compounds of yacon leaves may contribute to its functional effectiveness.

To assess the health benefits of processed yacon leaves grown in Japan, we embarked on an examination of the multi-functional role of commercial yacon herbal tea, especially on

Abbreviations: RSM, response surface methodology; CCD, central composite design; O₂⁻, superoxide anion; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS⁺, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) cation; PMS, phenazine methosulfate; NBT, nitroblue tetrazolium; FRAP, ferric reducing antioxidant capacity; TE, trolox equivalent; TPC, total phenolic content.

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its anti-inflammatory effects on macrophage cells (Ueda *et al.*, 2017a) and its inhibitory effects on digestive enzymes, including α -glucosidase and α -amylase (Ueda *et al.*, 2017b). Our previous report demonstrated the antioxidant effects of yacon herbal tea on multiple free radical scavenging assays, especially superoxide anion (O_2^-) radical generation systems (Sugahara *et al.*, 2015). Two-step heat-processing can effectively enhance the antioxidant activity in yacon leaves and alter levels of phenolic constituents, including caffeic acid (Ueda *et al.*, 2018). Among these reports, herbal tea extracts were prepared using hot-water at 90–100 °C for 45 min (Sugahara *et al.*, 2015; Ueda *et al.*, 2017a; 2017b) or 50 % methanol at 4 °C for 3 days (Ueda *et al.*, 2018). We were interested in examining the optimum extraction conditions to ensure higher antioxidant activities and total phenolic content (TPC) under hot-water extraction intended for normal drinking purposes.

Excess oxidative stress and reactive oxygen species, *e.g.*, O_2^- radical, hydroxyl radical, perhydroxyl radical, hydrogen peroxide, and singlet oxygen, have been implicated in the deleterious effects on normal tissue functions, thereby leading to pathological conditions (Gutowski and Kowalczyk, 2013). O_2^- radical has been implicated in a variety of pathological processes, such as diabetes, ischemia-reperfusion injury and chronic heart failure (Pacher *et al.*, 2006). Since superoxide dismutase (SOD) can decrease the O_2^- level in the human body, suppression of O_2^- can be considered as a strategy for screening SOD-like food materials and/or compound(s) to mitigate these disease risks. In our recent studies, commercial yacon leaf tea, and moringa leaf and stem herbal teas exhibited stronger O_2^- radical scavenging capacity than trolox (Sugahara *et al.*, 2015; 2018). Several representative polyphenols can demonstrate SOD-like O_2^- radical scavenging capacity, *e.g.*, phenolic acids including caffeic, chlorogenic and gallic acids, and flavonoids such as epicatechin, kaempferol, myricetin, quercetin and rutin (Sugahara *et al.*, 2018). Ferric reducing capability is a target in determining the antioxidant activity of test samples as well as biological fluids such as plasma (Benzie and Strain, 1996; 1999). Different free radical scavenging assays with synthetic 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical (Blois, 1958) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) cation (ABTS⁺) radical (Thaipong *et al.*, 2006), as well as ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1999) have been used to quantitatively evaluate and/or screen the antioxidative effect of test samples. Trolox has been used as a standardized antioxidant; thus, trolox equivalent (TE) value and/or trolox equivalent antioxidant capacity (TEAC) of test samples have been investigated in a variety of antioxidant assays.

In this study, we aimed to determine the optimum condition to obtain higher antioxidant activity in four parameters (DPPH, ABTS⁺, SOD and FRAP) and TPC during hot-water extraction of yacon tea. Response surface methodology (RSM) was

employed for extraction optimization by central composite design (CCD). According to the design methodology, RSM with CCD has been utilized to determine the effect of several factors one by one and also the majority of the variables (Jambrak, 2011). The two independent variables investigated here were extraction temperature (ranging from 75.0 to 96.0 °C) and time (from 2.00 to 5.50 min). Based on the fitted quadratic response surface model by RSM, the desirability value was determined to obtain optimum responses (Derringer and Suich, 1980).

Materials and Methods

Materials Dried herbal tea leaves from the yacon plant, locally cultivated, processed, and sold in the Kikuchi area (Kumamoto, Japan), were obtained in January 2018 in a retail store. The chemicals DPPH, ABTS, phenazine methosulfate (PMS), nitroblue tetrazolium (NBT), NADH, tripyridyltriazine (TPTZ), and Trolox were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethylsulfoxide was purchased from Merck KGaA (Darmstadt, Germany). Folin-Ciocalteu's reagent was purchased from Carlo Erba Reagents srl. (Cornaredo MI, Italy). Gallic acid was purchased from Fujifilm Wako Chemicals USA, Inc. (Richmond, VA, USA). All other chemicals were of the highest grade commercially available.

Preparation of hot-water extracts Hot-water extracts of yacon herbal tea were prepared in a shaking water-bath as follows. Approximately 1 g of crushed tea leaves (dried weight) was soaked in 50 mL of MilliQ water in a conical beaker at 75.0–96.0 °C for 2.00–5.50 min. The temperature was directly monitored using a hand-held digital thermometer. According to the CCD experimental design model in Design-Expert 7.0 (Stat-Ease Inc., Minneapolis, MN, USA), 13 runs with coded temperature X_1 and time X_2 for individual extraction were carried out as arranged in Table 1. Prepared extracts were kept at 4 °C until further experiments.

DPPH radical scavenging assay DPPH radical scavenging activity was measured as described by Blois (1958). The reaction was started by adding 0.5 mM DPPH dissolved in ethanol (50 μ L) to the pre-assay mixture (200 μ L) containing varying concentrations of the test sample (10 μ L), 70 % ethanol (90 μ L), and 0.1 M sodium acetate buffer (pH 5.5, 100 μ L). The reaction, in a final volume of 250 μ L, was allowed to proceed for 30 min at room temperature. The absorbance of the resulting solution was measured at 517 nm using the EZ Read 2000 Microplate Reader (Biochrom Ltd., Cambridge, UK). Trolox was used as a control standard. Activity data (%) obtained were converted to trolox equivalent (TE) values. To determine whether photometric absorption of samples/reagents interfered with the data, a parallel experiment at each point was carried out to evaluate the background signal.

ABTS⁺ radical scavenging assay ABTS⁺ radical scavenging activity was measured based on the method of Thaipong *et al.* (2006). ABTS-mixture solution was first

prepared by mixing equal volumes of 7.4 mM ABTS and 2.6 mM potassium peroxydisulfate solutions by rotation, for 15 h in the dark at room temperature. The ABTS-working solution was then prepared by diluting the ABTS-mixture solution (150 μ L) in methanol (2.9 mL). The reaction was started by adding ABTS-working solution (190 μ L) to varying concentrations of a test sample (10 μ L). The reaction, in a final volume of 200 μ L, was allowed to proceed at room temperature for 2 h in the dark. Absorbance of the resulting solution was measured at 734 nm. Trolox was used as a control standard. Activity data (%) obtained were converted to TE values.

O₂⁻ radical scavenging assay (SOD assay) O₂⁻ radical scavenging activity was measured using the phenazine methosulfate (PMS)-NADH-nitroblue tetrazolium (NBT) system according to previously described methods (Gulcin 2006; Wang *et al.*, 2008a). The reaction was started by adding 2 mM NADH (20 μ L) to the pre-assay mixture (180 μ L) containing varying concentrations of a test sample (10 μ L), 1 mM NBT (20 μ L), 0.1 mM PMS (20 μ L), 250 mM potassium phosphate buffer (pH 7.4, 40 μ L), and water (90 μ L). The reaction, in a final volume of 200 μ L, was allowed to proceed for 10 min at room temperature. Absorbance of the resulting solution was measured at 570 nm. Trolox was used as a control standard. Activity data (%) obtained were converted to TE values.

FRAP assay The FRAP assay was measured using TPTZ based on the method of Benzie and Strain (1996; 1999). The reaction was started by adding FRAP reagent (240 μ L) containing 0.3 M sodium acetate buffer (pH 3.6, 200 μ L), 10 mM TPTZ in 0.04 M HCl (20 μ L), and 20 mM FeCl₃ in MilliQ solution (20 μ L) to varying concentrations of a test sample (12.6 μ L). The reaction, in a final volume of 252.6 μ L, was allowed to proceed for 4 min at room temperature. Absorbance of the resulting solution was measured at 593 nm. Trolox was used as a control standard. Activity data (%) obtained were converted to TE values.

Determination of total phenolic content The amount of total phenols, including polyphenols, was determined by an established procedure (Singleton and Rossi, 1965). The mixture containing test samples (25 μ L) and 10-fold diluted Folin-Ciocalteu phenol reagent solution (125 μ L) was incubated with 10 % sodium carbonate solution (125 μ L) for 10 min at room temperature. The assay mixture was subjected to colorimetric measurement at 600 nm. Gallic acid was used as a control standard to prepare a calibration curve.

Experimental design and statistical analysis RSM using a CCD was applied as the experimental design to optimize the combination of temperature (ranging from 75.0 to 96.0 °C) and time (ranging from 2.50 to 5.00 min) in the hot-water extraction. The five coded levels were incorporated into the design for temperature -1.41 (75.0 °C), -1 (78.0 °C), 0 (86.0 °C), 1 (93.0 °C), 1.41 (96.0 °C) and time -1.41 (2.00 min), -1 (2.50 min), 0 (3.75 min), 1 (5.00 min), 1.41 (5.50 min) as

summarized in Table 1. The central point of the design (0,0) in cube was repeated five times. In total, 13 run designs, four cube points and four other axial points were included. The effect of the two independent variables on four different antioxidant activities and TPC was investigated using a second-order model. The second-order model equation used to fit responses and further analyses followed a previous report (Puttongsiri and Haruenkit, 2010) with the following equation.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad \dots \text{Eq. 1}$$

where Y is the predicted response value (DPPH, FRAP, SOD, ABTS⁺ and TPC), X₁ is temperature and X₂ is time, β_0 is a constant value, β_1 and β_2 are linear terms, β_{11} and β_{22} are quadratic terms, and β_{12} is an interaction term.

Design-Expert (version 7.0, Stat-Ease Inc.) was used to fit the model, draw the RSM cube-shape figure and determine desirability. Evaluation of the correlations among individual parameters was carried out in part using Pearson's correlation test with a statistical add-on software program (Statcel 4, OMS Co., Saitama, Japan). The experiment values are expressed as mean $\pm$ standard deviation derived from three parallel experiments.

Results and Discussion

To determine the optimum conditions for obtaining higher antioxidant activity and TPC during hot-water extraction of yacon tea leaves, RSM was used for the optimization of extraction by CCD. The prediction of the optimum conditions was performed using Design-Expert software.

Determination of the optimal level of response variables The RSM involving a CCD was performed with 13 experiments for the two factors of extraction temperature and time. The combination of temperature (X₁) and time (X₂) in each formulation and the response values based on four different antioxidant activities, including DPPH, ABTS⁺, SOD and FRAP, and TPC, are summarized in Table 1. Intended for normal drinking purposes, extraction temperature ranging from 75.0 to 96.0 °C and time ranging from 2.00 to 5.50 min were chosen. Notably, there was a trend for higher antioxidant activities and TPC when the extraction was performed at a higher temperature range (86.0-96.0 °C), while lower values were observed at a lower temperature range (75.0-78.0 °C). By a comparison of the minimum and maximum activity data obtained, a 1.80-times difference was observed in DPPH radical scavenging assay. For the ABTS⁺ radical scavenging, SOD and FRAP assays, and TPC determination, differences of 1.71-, 3.36-, 2.11-, and 1.88-times were observed, respectively. It is also of interest the kinds of antioxidants that can be extracted efficiently under this hot-water extraction setting. We recently examined the phenolic constituents of yacon leaves and reported the increase of caffeic acid level during heat-processing using high-performance liquid chromatography (HPLC) (Ueda *et al.*, 2018). A pilot HPLC experiment using

Table 1. Central composite design and experimental response values in the hot-water extraction of yacon herbal tea.

Run	Coded		Uncoded		Response				
	X ₁	X ₂	Temp (°C) (X ₁)	Time (min) (X ₂)	DPPH (µg TE/mL) (Y ₁)	ABTS ⁺ (µg TE/mL) (Y ₂)	SOD (µg TE/mL) (Y ₃)	FRAP (µg TE/mL) (Y ₄)	TPC (mg GAE/mL) (Y ₅)
1*	0	0	86.0	3.75	23.5±0.9	131±10	1,288±153	40.2±0.7	0.540±0.027
2*	0	0	86.0	3.75	29.1±2.0	107±6	1,293±263	45.8±0.7	0.633±0.013
3	0	1.41	86.0	5.50	29.9±1.5	122±10	1,373±199	46.1±1.6	0.611±0.051
4*	0	0	86.0	3.75	24.3±1.1	113±15	875±205	41.4±1.6	0.523±0.026
5	1	-1	93.0	2.50	30.3±2.8	111±6	1,098±204	43.1±0.8	0.632±0.023
6	1.41	0	96.0	3.75	30.7±2.4	111±1	1,343±119	44.9±0.5	0.627±0.014
7*	0	0	86.0	3.75	26.0±2.0	96.1±7.8	1,233±256	37.2±0.3	0.554±0.013
8*	0	0	86.0	3.75	29.3±0.7	117±7	1,721±205	38.5±1.6	0.605±0.017
9	-1.41	0	75.0	3.75	17.1±2.4	76.4±8.0	512±143	23.1±1.6	0.421±0.039
10	0	-1.41	86.0	2.00	23.6±1.6	115±11	1,232±143	39.8±2.2	0.540±0.013
11	1	1	93.0	5.00	30.5±1.1	118±8	1,400±182	40.4±0.4	0.602±0.034
12	-1	1	78.0	5.00	17.3±1.6	89.6±21.9	748±212	21.8±0.4	0.336±0.008
13	-1	-1	78.0	2.50	17.4±2.0	102±3	1,020±131	31.4±1.3	0.493±0.038
Max.					30.7	131	1,721	46.1	0.633
Ave.					25.3	108	1,164	38.0	0.547
Min.					17.1	76.4	512	21.8	0.336

*The central point of the design in cube. The five coded levels were incorporated as described (see Materials and Methods section). TE; trolox equivalent, GAE; gallic acid equivalent, Max.; maximum, Ave.; average, Min.; minimum.

Table 2. Correlations between the variable parameters obtained in this study.

	Correlation coefficient (<i>r</i>)				
	DPPH (µg TE/mL)	ABTS ⁺ (µg TE/mL)	SOD (µg TE/mL)	FRAP (µg TE/mL)	TPC (mg GAE/mL)
Temp (°C)	0.888***	0.628*	0.635*	0.806**	0.810***
Time (min)	0.173	0.0312	0.0741	-0.0450	-0.101
DPPH (µg TE/mL)	1.00	0.619*	0.770*	0.871***	0.920***
ABTS ⁺ (µg TE/mL)		1.00	0.744**	0.776**	0.655*
SOD (µg TE/mL)			1.00	0.708**	0.778**
FRAP (µg TE/mL)				1.00	0.927***
TPC (mg GAE/mL)					1.00

Significant at **P*<0.05, ***P*<0.01, or ****P*<0.001 using Pearson's correlation test.

another batch of commercial yacon herbal tea confirmed the presence of caffeic and chlorogenic acids in the hot-water extract (data not shown). To assess correlations between the two variables, regression analysis was performed next. Using Pearson's test, DPPH (*r* = 0.888), ABTS⁺ (*r* = 0.628), SOD (*r* = 0.635), FRAP (*r* = 0.806), and TPC (*r* = 0.810) parameters were positively and significantly correlated with the extraction temperature (Table 2). Notably, further significant correlations were shown among the four different antioxidant activities and TPC.

Extraction temperature and time are the essential

parameters that influence the ABTS⁺ radical scavenging and FRAP activities and phenol content in white tea (Burillo *et al.*, 2018). Wherein, there is a trend of increasing activity and phenol content when the extraction temperature (70, 80, and 90 °C) and time (3 and 5 minutes) increase; however, there is almost no change with the extraction at 80-90 °C under this experimental setting. In the case of green tea, the FRAP activity increases from 70 to 90 °C extraction, and the first 15 s of extraction greatly contributes to the activity (Langley 2000). Polyphenols in these herbal teas and/or green tea can play an important role in the antioxidant activity, while the active

Table 3. Analysis of variance of independent variables on the response variables for hot-water extraction of yacon herbal tea.

Source	df	F-value				
		DPPH	ABTS ⁺	SOD	FRAP	TPC
Model	5	8.25**	2.99	2.83	6.31**	4.46
X ₁ (Temp)	1	37.10***	8.13*	8.06*	23.86**	18.60**
X ₂ (Time)	1	1.47	0.30	0.12	0.07	0.29
X ₁ ²	1	2.68	6.05*	4.43	6.83*	2.19
X ₂ ²	1	0.04	0.08	<0.004	<0.004	0.08
X ₁ X ₂	1	<0.004	0.06	1.46	0.61	1.24
Residual Error	7	-	-	-	-	-
Lack of Fit	3	0.92	0.14	0.12	2.84	2.22
Pure Error	4	-	-	-	-	-
R ²	-	0.8549	0.6808	0.6689	0.8183	0.7613

Coded X₁ and X₂ indicate temperature and time, respectively. Significant at **P* < 0.05, ***P* < 0.01 or ****P* < 0.001. df, degree of freedom.

Table 4. Predictive models from the response surface methodology

Dependent values	Quadratic models	Eq.
DPPH	$Y_1 = -171 + 4.00X_1 + 0.930X_2 - 0.0202X_1^2 - 0.0922X_2^2 + 0.00556X_1X_2$	(2)
ABTS ⁺	$Y_2 = -885 + 22.3X_1 + 6.16X_2 - 0.123X_1^2 + 0.518X_2^2 - 0.100X_1X_2$	(3)
SOD	$Y_3 = -14,389 + 382X_1 - 868X_2 - 2.34X_1^2 - 0.833X_2^2 + 10.6X_1X_2$	(4)
FRAP	$Y_4 = -374 + 9.50X_1 - 11.3X_2 - 0.0541X_1^2 + 0.0400X_2^2 + 0.128X_1X_2$	(5)
TPC	$Y_5 = -2.35 + 0.0678X_1 - 0.185X_2 - 0.000395X_1^2 - 0.00278X_2^2 + 0.00235X_1X_2$	(6)

X₁ = temperature (°C)
X₂ = time (min)
Eq., equation.

constituents yielded may depend on the extraction design. Notably, short-term extraction of a fruit can provide optimized phenolic content and antioxidant activity using RSM (Belwal *et al.*, 2016). In contrast, there are some reports that long-term extraction leads to undesired oxidation of polyphenols (Belwal *et al.*, 2016; Naczki and Shahidi, 2004).

To visualize the general extraction process, each response was next confirmed by applying the second order polynomial equation to each variable. Table 3 shows the ANOVA (analysis of variance) of two independent coded variables, temperature and time, vs. five dependent variables, DPPH, ABTS⁺, SOD and FRAP, and TPC. Predictive quadratic models from RSM in dependent parameters are shown as equations in Table 4. Based on the respective *F* values, models of DPPH and FRAP could significantly predict the effects of extraction temperature and time as the parameters associated with the antioxidative functionality of yacon herbal tea. In individual cases, temperature (X₁) showed significant correlations with all the values from DPPH, ABTS⁺, SOD, FRAP, and TPC, while time (X₂) showed no significant correlation. Interestingly, the quadratic effect of temperature (X₁²) was demonstrated only for

ABTS⁺ and FRAP, while no quadratic (X₂²) effect of time and no interactive effects between temperature and time (X₁X₂) were observed. It should be emphasized that these models are adequate because there was no significant lack of fit in any of the response variables. The coefficient values (R²) of all models ranged between 66.9 and 85.5 %, while no residual and pure errors on ANOVA were observed.

Figure 1A to 1E shows the visualized response surface plots of independent variables on DPPH, ABTS⁺, SOD, FRAP, and TPC, respectively. DPPH radical scavenging capacity increased when the extraction temperature increased (Figure 1A, also see Table 4 (Eq. 2)). Neither temperature nor time showed a quadratic effect on the DPPH value as its concentration increased. Temperature appeared to have a greater effect on the DPPH value than did time. The current experiment confirmed that extraction temperature could increase the DPPH radical scavenging capacity of yacon herbal tea. With increasing extraction temperature, the values of the ABTS⁺ radical scavenging capacity (Figure 1B, also see Table 4 (Eq. 3)), SOD-like capacity (Figure 1C, also see Table 4 (Eq. 4)), FRAP activity (Figure 1D, also see Table 4 (Eq. 5)), and

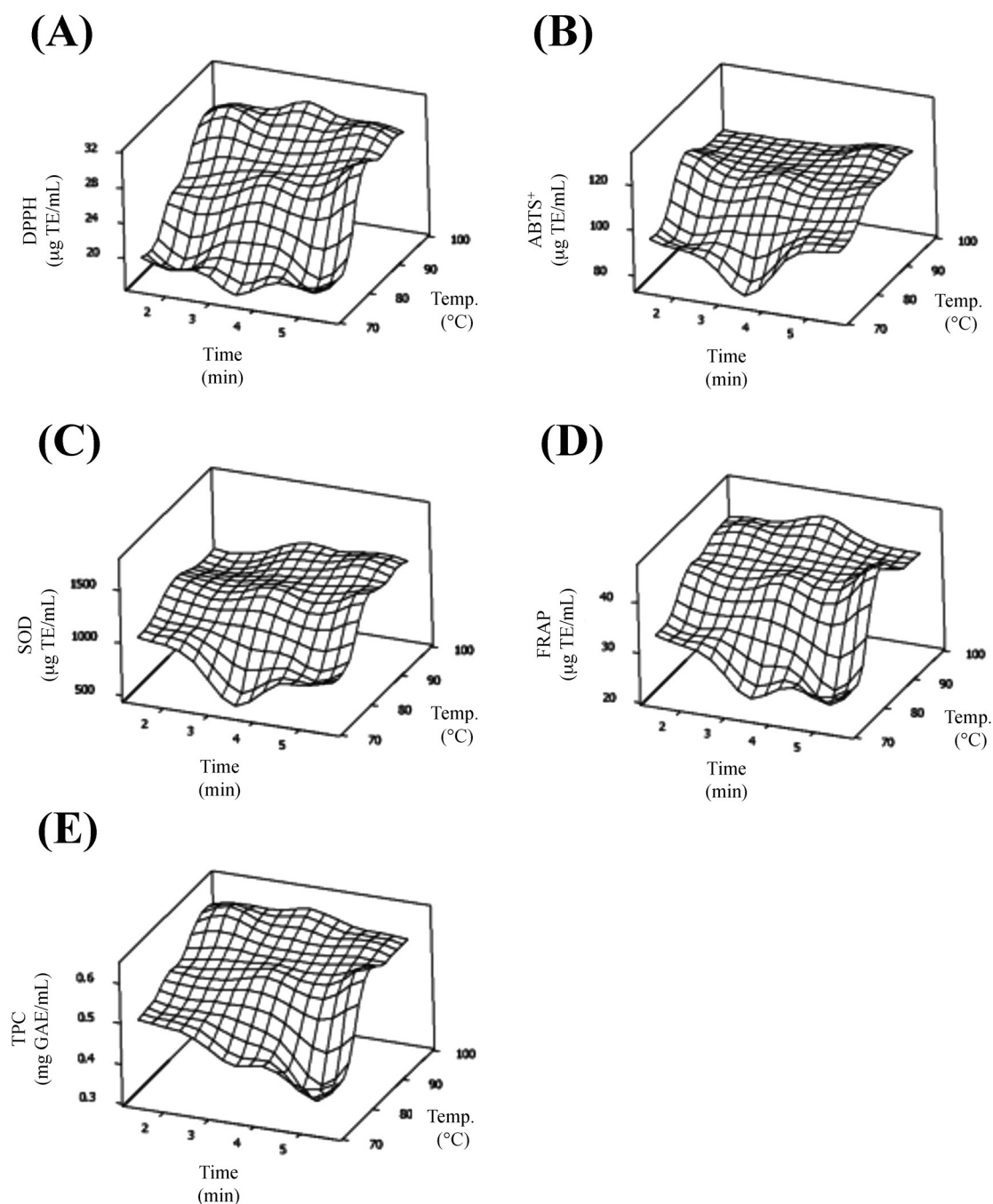


Fig. 1. Response surface plots of extraction temperature and time on four antioxidant 5 activities, including DPPH (A), ABTS⁺ (B), SOD (C), and FRAP (D), and TPC (E) of 6 yacon herbal tea leaves. TE; trolox equivalent, GAE; gallic acid equivalent.

TPC (Figure 1E, also see Table 4 (Eq. 6)) were also concomitantly elevated. There was a trend that these antioxidant capacities and phenolic content reached maximum around 90–100 °C. In previous reports, an increase of extraction temperature was shown to contribute to the extraction efficiency of active polyphenol compounds from plant materials (Belwal *et al.*, 2016; Tan *et al.*, 2013; Wang *et al.*, 2008b). There is a correlation between tea polyphenol contents

and DPPH radical scavenging assay (Turkmen *et al.*, 2006), as well as ABTS/FRAP assays (Liu *et al.*, 2009). Our varying activity data and TPC at different extractions from yacon herbal tea may have resulted from differences in the extraction efficiencies of the extracted active polyphenols.

Optimization of temperature and time Optimization was based on the generation of results that maximized the response for DPPH, ABTS⁺, SOD, FRAP, and TPC. The desirability

Table 5. Optimization of extraction temperature and time in terms of composite properties.

Response variable	Optimization of extraction temperature and time					
	Goal	Lower	Upper	Target	Predicted responses	Desirability
DPPH ($\mu\text{g TE/mL}$)	Maximize	17.1	30.7	30.7	30.3	0.968
ABTS ⁺ ($\mu\text{g TE/mL}$)	Maximize	76.4	131	131	117	0.737
SOD ($\mu\text{g TE/mL}$)	Maximize	512	1,721	1,721	1,443	0.770
FRAP ($\mu\text{g TE/mL}$)	Maximize	21.8	46.1	46.1	43.0	0.872
TPC (mg GAE/mL)	Maximize	0.336	0.633	0.633	0.634	1.00
Extraction temperature = 89.3 °C, Extraction time = 2.50 min						
Composite desirability = 0.863						

function value varied from 0 (undesirable response) to 1 (desirable response). The desirability function was based on transforming the measured property of each response to a dimensionless desirability scale. The optimum desirability range has been proposed to be 0.80-0.63 (Lazic, 2004; Puttongsiri and Haruenkit, 2010). The desirability values for the parameters tested in this study were 0.737 to 1.00, while the composite desirability was determined to be 0.863 (Table 5). Therefore, the optimum temperature and time for extraction were found to be 89.3 °C and 2.50 min, respectively. Notably, the predicted values of DPPH, ABTS⁺, SOD, FRAP, and TPC were 30.3 $\mu\text{g TE/mL}$, 117 $\mu\text{g TE/mL}$, 1,443 $\mu\text{g TE/mL}$, 43.0 $\mu\text{g TE/mL}$, and 0.634 mg GAE/mL, respectively. Additionally, only TPC showed a desirability value of 1.00 at this optimized extraction. These results support that RSM with CCD is an applicable tool for determining the optimum extraction settings of yacon tea, even within the narrow ranges of temperature and time. We must note that this method, RSM with CCD, has been used by researchers to determine optimum conditions for efficient extraction of targeted chemicals exhibiting higher functional activities from several other plants. For example, the optimum condition for efficient extraction of rosmarinic and carnosic acids and carnosol from rosemary is ethanolic-water extraction for 55 min (Oliveira *et al.*, 2016). The optimum condition for high TPC from Henna leaves is 48.07 % acetone extraction for 73.87 min at 39.57 °C (Uma *et al.*, 2010), while that of high antioxidant activities with higher amounts of epigallocatechin gallate from green tea (Kim *et al.*, 2016) is 57.7 % ethanol extraction for 15 min at 70 °C. To gain insight into the optimum industrial setting for the extraction of yacon herbal tea, wider ranges of time and temperature could be further investigated in future research.

In our previous report, the antioxidant activities of yacon tea per 100 g dry weight determined in tea leaf are 7.56 g TE in DPPH assay, 10.5 g TE in ABTS⁺ assay, and 208 g TE in O₂⁻ radical scavenging (SOD) assay, while TPC has 7.76 g chlorogenic acid equivalent (Sugahara *et al.*, 2015). In this study, the calculated antioxidant activities per 100 g dry weight determined in tea leaf at the optimum setting are 0.152 g TE in

DPPH assay, 0.585 g TE in ABTS⁺ assay, and 7.22 g TE in SOD assay, while TPC shows 3.17 g gallic acid equivalent. These values are much lower than those demonstrated in the previous report. Presumably, these differences may be due to differences in product batches and extraction condition; for example, our previous report involved three sequential hot-water extractions at 90-100 °C for 45 min (Sugahara *et al.*, 2015). In addition, the composition and/or amount of yielded active constituents may vary during different extraction conditions. Nevertheless, the difference between the data of the previous paper and our current study may result from the harvest season, year, cultivation environment, cultivar, and/or drying process such as sun drying versus hot-air drying. Further research is needed to clarify this point. Interestingly, a TE value of more than 1.00 was found in the SOD assay, implying that the antioxidant capacity of SOD is higher than that observed for trolox as the positive standard in both studies. We previously demonstrated the anti-inflammatory effects (Ueda *et al.*, 2017a) and inhibitory activities on α -glucosidase and α -amylase of yacon tea (Ueda *et al.*, 2017b). Whether the optimization methodology can provide more suitable extraction conditions for humans, thereby resulting in health benefits, is also another interesting research question.

Conclusion

Based on the fitted quadratic model by RSM, optimized extraction of yacon tea was achieved at 89.3 °C for 2.50 min (desirability value of 0.863) to obtain higher antioxidant activity and phenolic content. Here, we for the first time attempted to investigate whether regular tea extraction with narrow ranges of temperature (from 75.0 to 96.0 °C) and time (from 2.00 to 5.50 min) can provide the optimum condition for higher antioxidant activity and TPC. To establish yacon herbal tea as a foodstuff with health benefits, we focused here on determining the best extraction conditions from the viewpoint of a traditional brewed tea usage scenario. Nevertheless, RSM with CCD analysis is a methodology designed for obtaining optimum conditions in a large-scale industrial setting. Determining the extraction conditions that can provide yacon

extracts with much higher antioxidant levels and other health benefits is an important research question in the context of large scale industrial production of this herbal tea. More studies are needed to fully achieve this goal.

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Disclosure statement

No potential conflicts of interest were disclosed.

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