Optimization of roasting conditions through antioxidant and anti-inflammatory activities of *Yak-kong* (*Rhynchosia nulubilis*)

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Abstract The effects of roasting conditions on the antioxidant activities and anti-inflammatory activities of roasted *yak-kong* were investigated using a second-order central composite design. The optimum conditions for DPPH radical scavenging ability (IC_{50}), ABTS radical scavenging activity (IC_{50}), total phenolic content (TPC), and oxygen radical absorbance capacity (ORAC) were at 111.47°C for 20.45 min, with the best values (DPPH, IC_{50} ; 2.143 mg/mL, ABTS, IC_{50} ; 1.775 mg/mL, TPC; 51.39 mg tannic acid (TAE)/g, and ORAC; 6.89 μ mol trolox equivalents (TE)/g). The optimum conditions of nitric oxide (NO) production, prostaglandin E2 (PGE2) production, and tumor necrosis factor- α (TNF- α) were at 110.24°C for 21.18 min, yielding the best values (NO; 14.484 μ M, PGE2; 3.433 mg/mL, and TNF- α ; 3.818 ng/mL). Superimposed contour plots with regard to 7 variables indicated that the optimum roasting temperature and time were 110.88°C and 20.86 min. This result suggested that the optimally roasted *yak-kong* could replace coffee beans to provide potential bone health benefits to heavy coffee drinkers.

Keywords: *Yak-kong* (*seomoktae*), roasting, response surface methodology, antioxidant activity, anti-inflammatory activity

Introduction

Osteoporosis is a predominant health issue in postmenopausal women, wherein estrogen concentration decreases with increase in the rate of bone reformation (1). Increased bone reformation is related to decreased bone mineral density (BMD) (2). The annual decline rate of BMD in the hip and lumbar spine has been shown to be 1–2.4% in postmenopausal women (1).

Various factors including oxidative stress, hormones, and nutrition can affect bone remodeling (3,4). However, oxidative stress associated with free radical production and antioxidant defense pertaining to the activities of bone cells has received little attention (4,5).

Several epidemiological studies have been conducted to quantify the relationship between coffee consumption and the risk of fractures (6). In a recently published meta-analysis, Liu *et al.* (7) noted that the risk of fractures among coffee drinkers was high, particularly among postmenopausal women. As the coffee consuming population continues to grow annually (8), the prevalence of osteoporosis and osteoporosis-related fractures will likely continue to increase, contributing to a rapid growth in the social and economic burden in the near future (9).

The association between coffee consumption and the risk of fractures associated with it is important because osteoporosis and

osteoporosis-related fractures are major public health problems worldwide (10) also because coffee is one of the most widely consumed beverages around the world (11).

Therefore, it is necessary to find replacement goods for coffee beans to reduce osteoporosis. Phytoestrogens may slightly suppress osteoporosis in postmenopausal women (12). The most commonly ingested soy isoflavones have structural similarity with estrogen (13). Epidemiological study reports that women who consume dietary soy products have a low risk of osteoporosis (14). *Yak-kong* (*seomoktae*), a type of black soybean, contains natural phenolic compounds (15) such as isoflavones and polyphenols, which exert protective effects against osteoporosis in menopausal women (16) due to its antioxidant and anti-inflammatory functions (15,17). Few studies have focused on finding effective roasting conditions to improve both its anti-oxidative and anti-inflammatory properties to ameliorate bone loss (18,19).

Response surface methodology (RSM) is an effective statistical tool for optimizing complex processes, which is widely used for extraction process using phenolic compounds (20).

Therefore, this study was conducted to determine the optimal roasting condition for *yak-kong* with the intention of replacing coffee beans to provide potential bone health benefits to heavy coffee drinkers. RSM was used to determine the optimum roasting temperature

and roasting time for *yak-kong* (*seomoktae*, *Rhynchosia nulubilis*) with high anti-oxidation and anti-inflammation. *Yak-kong* samples, with sizes smaller than other black beans, were roasted at temperatures ranging from 90 to 130°C for 10–30 min.

Materials and Methods

Materials *Yak-kong* (*seomoktae*, *Rhynchosia nulubilis*) was purchased from Chorokmaeul Co. Inc. (Seoul, Korea) for roasting.

Chemicals DPPH, ABTS, Na₂CO₃, tannic acid, Folin-Ciocalteu reagent, potassium persulfate, and phosphate buffer saline were purchased from Sigma-Aldrich (St. Louis, MO, USA) and the ORAC activity assay kit was purchased from Cell Biolabs, Inc. (San Diego, CA, USA). All chemicals and reagents used were of analytical grade and obtained from commercial sources.

Roasting of *yak-kong* and preparation of its extract *Yak-kong* (500 g) was rinsed with water for 1 min and strained for 30 s to remove water. Five different roasting conditions (temperature: 90, 100, 110, 120, and 130°C; time: 10, 15, 20, 25, and 30 min) were set using a central composite design (CCD) of RSM. Roasting was performed using a roaster (Proaster THCR-01; Taehwan Automation Industry Co., Ltd., Bucheon, Korea). The roasted *yak-kong* was ground using a grinder (Caimano; ANFIM, Milan, Italy) and the resultant powder was extracted with water (1:20) for 3 h at 80°C using a heating mantle (HM250C; Sergrim Lab Tech, Seoul, Korea). The final extract was filtered through a 0.22 µm PIFE filter unit and concentrated at 40°C using a rotary vacuum evaporator (HS-2005S-N; Hahn Shin Scientific Co., Ltd., Bucheon, Korea) to obtain a final volume of 50 mL. Next, the samples were freeze dried (FD8508; Ilshin Biobase, Dongducheon, Korea) and then stored (70°C) in a freezer (WUF-500; Daihan Scientific, Wonju, Korea) until further analysis.

Experimental design This study was performed in accordance with previous studies (21,22); two independent variables, roasting temperature (X_1) and roasting time (X_2), were used and the experimental conditions for each are presented in Table 1, Table 2-1, and Table 2-2. The experimental conditions were determined based on preliminary experimental results. The two independent variables were coded in five levels each as 2, 1, 0, -1, and -2. The response variables for anti-oxidation were DPPH radical scavenging activity (Y_1), ABTS radical scavenging activity (Y_2), total polyphenol content (Y_3), and oxygen radical absorbance capacity (Y_4). The response variables for anti-inflammation were nitric oxide (NO) production (Y_5), PGE2 production (Y_6), and TNF- α (Y_7).

The experimental runs were randomized to minimize the effects of unexpected variability in the observed responses. The variables were coded according to the following equation:

Table 1. Independent variables (temperature and time) and their coded levels used for optimization

Independent variables	Symbol	Coded level				
		-2	-1	0	1	2
Temperature (°C)	X_1	90	100	110	120	130
Time (min)	X_2	10	15	20	25	30

$$X = (X_i - X_0) / \Delta X \quad (1)$$

where X is the coded value, X_i is the corresponding actual value, X_0 is the actual value in the center of the domain, and ΔX is the increment of X_i corresponding to a variation of 1 unit of X . The mathematical model corresponding to the composite design is

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (2)$$

where Y is the response variable. β_0 , β_1 , and β_{11} represent coefficient estimates where β_0 is the interception, β_1 is the linear term, and β_{11} is the quadratic term. Results were analyzed and predicted using the Design expert 8 (Stat-Ease Inc., Minneapolis, MN, USA) and the analysis of variance (ANOVA) method with RSM.

Determination of DPPH radical scavenging activity The DPPH radical scavenging activity of the roasted *yak-kong* extracts was measured following a modified protocol from Blois (23). A 100 µL aliquot sample and 100 µL of a 0.2 mM DPPH solution (Sigma-Aldrich) were allowed to react at room temperature for 30 min in the dark. The absorbance after 30 min was measured at 517 nm using an ELISA plate reader (infinite® F500; Tecan, Grodig, Austria). The capacity of the sample to scavenge the DPPH radical was calculated as

$$\text{DPPH radical scavenging (\%)} = [(A_0 - A_1) / A_0] \times 100 \quad (3)$$

where A_0 is the absorbance of the control reaction and A_1 is the absorbance of the sample reaction. The IC₅₀ value (concentration of extract that causes 50% scavenging) was determined from a graph of the scavenging effect percentage plotted against the extract concentration. The lower the IC₅₀ value, the higher the antioxidant power. All the determinations were performed three times.

Determination of ABTS radical scavenging effect The ABTS radical scavenging effect of the roasted *yak-kong* extracts is based on the effect of the antioxidants to reduce the ABTS radical and was conducted as described by Jeong *et al.* (24). ABTS was prepared by reacting 7.4 mM ABTS aqueous solution with 2.6 mM potassium persulfate in the dark for 24 h at room temperature. The resultant ABTS solution was diluted with 0.01 M phosphate buffer saline (PBS, pH 7.4) and the absorbance was adjusted to 0.70±0.02 at 732 nm using an ELISA plate reader (infinite® F500; Tecan). The absorbance was measured with a spectrophotometer at 732 nm against a blank after 5 min of reaction at room temperature. The capacity of the sample to scavenge the ABTS radical was calculated as

Table 2-1. Responses of dependent variables to reaction conditions for independent variables through antioxidant effect

No.	Coded independent variables		Independent variables (actual values)		Response variables ¹⁾ (experimental data)			
	X ₁	X ₂	Temperature (°C)	Time (min)	DPPH ¹⁾	ABTS	TPC	ORAC
1	-1	-1	100	15	2.691±0.026	2.317±0.024	47.012±0.38	3.053±0.081
2	1	-1	120	15	2.458±0.012	1.958±0.026	46.658±0.34	5.923±0.065
3	-1	1	100	25	2.496±0.019	2.266±0.041	48.533±0.42	4.925±0.072
4	1	1	120	25	2.701±0.024	1.984±0.015	45.191±0.36	5.695±0.049
5	-2	0	90	20	3.168±0.033	2.807±0.066	41.336±0.23	1.133±0.025
6	2	0	130	20	2.887±0.017	2.485±0.032	42.133±0.43	3.998±0.071
7	0	-2	110	10	2.401±0.014	2.022±0.011	48.034±0.28	3.655±0.054
8	0	2	110	30	2.324±0.016	2.113±0.029	50.641±0.33	6.006±0.053
9	0	0	110	20	2.037±0.058	1.701±0.018	51.062±0.25	6.697±0.068
10	0	0	110	20	2.069±0.042	1.767±0.021	52.009±0.39	6.719±0.079
11	0	0	110	20	2.011±0.025	1.757±0.034	52.573±0.37	7.003±0.087
12	0	0	110	20	2.133±0.032	1.811±0.017	51.943±0.57	6.842±0.112
13	0	0	110	20	2.201±0.027	1.786±0.036	51.037±0.49	7.097±0.094

¹⁾DPPH, ABTS radical scavenging activity (IC₅₀, mg/mL); TPC, total polyphenol content (mg tannic acid (TAE)/g) and ORAC, oxygen radical absorbance capacity (μmol trolox equivalents (TE)/g).

Table 2-2. Responses of dependent variables to reaction conditions for independent variables through anti-inflammatory effect

No.	Coded independent variables		Independent variables (actual values)		Response variables ¹⁾ (experimental data)		
	X ₁	X ₂	Temperature (°C)	Time (min)	NO ¹⁾	PGE2	TNF-α
1	-1	-1	100	15	20.017±0.348	3.997±0.018	4.312±0.037
2	1	-1	120	15	17.293±0.354	3.778±0.015	4.155±0.032
3	-1	1	100	25	18.928±0.136	3.746±0.019	4.104±0.014
4	1	1	120	25	19.084±0.218	3.870±0.011	4.242±0.027
5	-2	0	90	20	25.096±0.414	4.301±0.022	4.534±0.009
6	2	0	130	20	22.779±0.292	4.169±0.013	4.505±0.042
7	0	-2	110	10	18.057±0.153	3.949±0.035	4.394±0.053
8	0	2	110	30	16.609±0.274	3.868±0.017	3.910±0.025
9	0	0	110	20	13.474±0.127	3.375±0.029	3.861±0.017
10	0	0	110	20	14.768±0.254	3.431±0.018	3.875±0.021
11	0	0	110	20	14.012±0.299	3.407±0.022	3.713±0.036
12	0	0	110	20	14.591±0.322	3.453±0.014	3.805±0.045
13	0	0	110	20	13.569±0.375	3.341±0.034	3.724±0.057

¹⁾NO, nitric acid (μM); PGE2, prostaglandin E2 (mg/mL); TNF-α, tumor necrosis factor-α (ng/mL).

$$\text{ABTS radical scavenging (\%)} = [(A_0 - A_1)/A_0] \times 100 \quad (4)$$

where A₀ is the absorbance of the control reaction and A₁ is the absorbance of the sample reaction. IC₅₀ values were measured from a graph of the scavenging activity percentage plotted against the extract concentration. The lower the IC₅₀ value, the higher the antioxidant power. All the determinations were performed three times.

Determination of total phenolic and total polyphenol contents (TPC) The TPC content of the roasted *yak-kong* extracts was determined following the method introduced by Materska and Perucka (25). Samples (1,000 μg/mL) or tannic acid (0-0.1 mg/mL)

was mixed with 20 μL of 1 N Folin-Ciocalteu reagent. This solution was mixed with 100 μL of 2% Na₂CO₃. The mixture was incubated on a shaker at room temperature for 30 min. The absorbance was measured at 725 nm. A standard curve was established with various tannic acid concentrations.

Oxygen radical absorbance capacity (ORAC) An OxiSelect™ ORAC activity assay kit (Cell Biolabs, Inc.) was used for determination of ORAC values of the roasted *yak-kong* extracts (26). For the analysis, the sample (25 μL) was mixed with a fluorescein solution (150 μL) and incubated for 30 min. Subsequently, 25 μL of 2,2-azobis (2-amidinopropane) dihydrochloride solution in phosphate buffered saline (80 mg/mL) that contained 0.02% (w/v) KCl, 0.8% (w/v) NaCl,

0.02% (w/v) KH_2PO_4 , and 0.29% (w/v) $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ was added to the solution. ORAC assay was immediately conducted on a fluorescence microplate reader (Mithras LB940; Berthold, Bad Wildbad, Germany) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. The area under the fluorescence decay curve (AUC) was calculated by point-to-point integration. A decrease in fluorescence was determined at 5 min intervals for 60 min. The protective activity of an antioxidant in the sample was determined by comparing the AUC of the sample with that of a known antioxidant, Trolox, which is a water-soluble analog of Vitamin E. The ORAC values were represented as μmol Trolox equivalents (TE) per gram of samples.

Measurement of nitric acid (NO) production NO production of the roasted *yak-kong* extracts was determined by assessment of nitrite concentration in the supernatant of cultured RAW 264.7 cells with Griess reagent. Briefly, cells at a density of 1×10^5 cells/well were cultured in a 96-well plate, and then stabilized for 24 h under 5% CO_2 at 37°C . Cells were washed with PBS, replaced with fresh media, and incubated with $1 \mu\text{g/mL}$ LPS in the presence or absence of roasted *yak-kong* extract at $100 \mu\text{g/mL}$. After an additional 24 h incubation, the media were collected and nitrite concentration was analyzed (13). The absorbance was determined at 570 nm (Vision Scientific, Daejeon, Korea) and nitrite concentration was calculated.

Measurement of prostaglandin E2 (PGE2) production Raw 264.7 cells were cultured in 24-well plates then incubated with or without $1 \mu\text{g/mL}$ LPS in the absence or presence of the *yak-kong* extracts at $100 \mu\text{g/mL}$. After 24 h incubation, the PGE2 concentration in the culture medium was determined with an enzyme linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Measurement of tumor necrosis factor- α (TNF- α) production Raw 264.7 cells were cultured in 24-well plates then incubated with or without $1 \mu\text{g/mL}$ LPS in the absence or presence of the *yak-kong* extracts at $100 \mu\text{g/mL}$. After 24 h incubation, the TNF- α concentration in the culture medium was determined with solid phase ELISA (R&D Systems). All incubation steps were conducted at room temperature. The optical density was measured with an ELISA plate reader (infinite® F500; Tecan) at 450 nm and corrected at the reference wavelength of 570 nm.

Statistical analysis Experimental data were statistically analyzed using the Design-expert 8 (Stat-Ease Inc.) software. ANOVA was performed and the fitness of the polynomial model equation was evaluated using coefficient of regression (R^2) and the F -value. The significances of all terms in the polynomial were considered statistically different when $p < 0.05$. The adequacy of the model was verified by accounting for the coefficient of determination (R^2).

Results and Discussion

The effects of the 2 variables [temperature (X_1) and time (X_2)] on the optimum conditions for *yak-kong* roasting were determined through effects of anti-oxidation and anti-inflammation. The 4 response variables evaluated for anti-oxidation were DPPH radical scavenging activity, ABTS radical scavenging activity, TPC, and ORAC. The results of 13 runs made with a CCD design are shown in Table 2-1, which includes the values for the observed responses. The minimum DPPH radical scavenging activity (IC_{50} : 2.011 mg/mL) was observed at $X_1 = 110^\circ\text{C}$, $X_2 = 20$ min. The minimum ABTS radical scavenging activity (IC_{50} : 1.701 mg/mL) occurred at $X_1 = 110^\circ\text{C}$ and $X_2 = 20$ min. The maximum TPC (52.573 mg TAE/g) was at $X_1 = 110^\circ\text{C}$ and $X_2 = 20$ min and the maximum ORAC value (7.097 μmol TE/g) was at $X_1 = 110^\circ\text{C}$ and $X_2 = 20$ min.

The 3 response variables evaluated for anti-inflammation were NO production, PGE2 production, and TNF- α . The results of 13 runs made with a CCD design are presented in Table 2-2, which includes the values for the observed responses. The minimum NO production (13.474 μM) was at $X_1 = 110^\circ\text{C}$ and $X_2 = 20$ min. The minimum PGE2 production (3.341 mg/mL) occurred at $X_1 = 110^\circ\text{C}$ and $X_2 = 20$ min and the minimum TNF- α (3.713 ng/mL) was at $X_1 = 110^\circ\text{C}$ and $X_2 = 20$ min.

The results of quadratic models for the data are presented in Table 3. A quadratic model with the interaction terms between roasting temperature and roasting time was used for verification of the significance of the probability values. The probabilities of the DPPH radical scavenging effect (IC_{50}), ABTS radical scavenging effect (IC_{50}), TPC, ORAC, NO, PGE2, and TNF- α were $p < 0.0017$, $p < 0.0001$, $p < 0.0004$, $p < 0.0001$, $p < 0.0002$, $p < 0.0001$, and $p < 0.0018$, respectively. In the model equations for DPPH radical scavenging activity, the constants for temperature and time were 0.572 and 0.357, respectively. Thus, DPPH radical scavenging effect was more dependent on

Table 3. Second-order polynomial equations for DPPH, ABTS, TPC, ORAC, NO, PGE2, and TNF- α

Responses	Model	Equations in terms of coded factors ¹⁾	R^2	p -value
Y_1 (DPPH)	Quadratic	$37.44 - 0.572X_1 - 0.357X_2 + 2.19 \times 10^{-3}X_1X_2 + 2.376 \times 10^{-3}X_1^2 + 2.857 \times 10^{-3}X_2^2$	0.9071	<0.0017
Y_2 (ABTS)	Quadratic	$31.80 - 0.506X_1 - 0.163X_2 + 3.85 \times 10^{-4}X_1X_2 + 2.216 \times 10^{-3}X_1^2 + 3.08 \times 10^{-3}X_2^2$	0.9697	<0.0001
Y_3 (TPC)	Quadratic	$-330.20 + 6.140X_1 + 4.315X_2 - 0.03X_1X_2 - 0.025X_1^2 - 0.025X_2^2$	0.9381	0.0004
Y_4 (ORAC)	Quadratic	$-166.37 + 2.671X_1 + 2.088X_2 - 0.011X_1X_2 - 0.011X_1^2 - 0.021X_2^2$	0.9823	<0.0001
Y_5 (NO)	Quadratic	$368.08 - 5.824X_1 - 2.961X_2 + 0.014X_1X_2 + 0.025X_1^2 + 0.034X_2^2$	0.9484	0.0002
Y_6 (PGE2)	Quadratic	$35.15 - 0.5X_1 - 0.4X_2 + 1.715 \times 10^{-3}X_1X_2 + 2.103 \times 10^{-3}X_1^2 + 5.148 \times 10^{-3}X_2^2$	0.9647	<0.0001
Y_7 (TNF- α)	Quadratic	$31.16 - 0.434X_1 - 0.327X_2 + 1.475 \times 10^{-3}X_1X_2 + 1.833 \times 10^{-3}X_1^2 + 3.689 \times 10^{-3}X_2^2$	0.9057	0.0018

¹⁾ X_1 , temperature; X_2 , time.

Table 4-1. Significance of regression (*F*-value, *p*-value) for anti-oxidation (DPPH radical scavenging effect (IC₅₀), ABTS radical scavenging effect (IC₅₀), TPC, and ORAC)

Source ¹⁾	DPPH		ABTS		TPC		ORAC	
	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value
Model	13.66	0.0017	44.84	<0.0001	21.21	0.004	77.65	<0.0001
$X_1^{1)}$	1.44	0.2686	24.26	0.0017	0.046	0.8362	71.46	<0.0001
X_2	0.047	0.8532	0.36	0.5663	0.29	0.6045	32.78	0.0007
X_1X_2	2.39	0.1663	0.26	0.6249	6.15	0.0423	10.77	0.0135
X_1^2	64.42	<0.0001	198.43	<0.0001	99.41	<0.0001	252.56	<0.0001
X_2^2	5.82	0.0467	23.95	0.0018	5.94	0.0450	59.80	0.0001

¹⁾ X_1 , temperature; X_2 , time.**Table 4-2.** Significance of regression (*F*-value, *p*-value) of equations for anti-inflammation (NO, PGE₂, and TNF- α)

Source	NO		PGE ₂		TNF- α	
	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value	<i>F</i> -value	<i>p</i> -value
Model	25.72	0.0002	38.22	<0.0001	13.45	0.0018
$X_1^{1)}$	3.73	0.0949	1.74	0.2289	0.036	0.8554
X_2	0.35	0.5750	1.39	0.2770	7.15	0.0319
X_1X_2	1.79	0.2231	4.76	0.0655	1.57	0.2500
X_1^2	122.36	<0.0001	164.03	<0.0001	55.70	0.0001
X_2^2	13.87	0.0074	61.41	0.0001	13.86	0.0074

¹⁾ X_1 , temperature; X_2 , time.

roasting temperature than on roasting time. Temperature also greatly affected ABTS radical scavenging (IC₅₀), TPC, ORAC, NO, PGE₂, and TNF- α .

The significance of regression for anti-oxidation and anti-inflammation is shown in Table 4-1 and Table 4-2. When the absolute *F*-value increased and the *p*-value decreased, the corresponding variables were more significant (27).

DPPH radical scavenging effect Although there are several methods available to determine the radical scavenging effects of antioxidants, the DPPH method is preferred because it is less time consuming, easy, reliable, and does not require any special device. DPPH is a stable, synthetic radical that does not disintegrate in water, methanol, or ethanol. The free radical scavenging effects of extracts depend on the effect of antioxidant compounds to lose hydrogen and the structural conformation of these components (25). Concerning the DPPH radical scavenging activity (IC₅₀), we observed that the quadratic terms for temperature (X_1^2) had the largest effects (Table 4-1) with a high regression coefficient ($R^2=0.9071$, $p<0.001$) (Table 3). However, the temperature (X_1) and time (X_2) were found not to have a significant difference (Table 4-1). The 3D response surface for DPPH radical scavenging effect (IC₅₀) is shown in Fig. 1A. The temperature greatly affected the DPPH scavenging effect (IC₅₀) and the roasting condition ($X_1=110^\circ\text{C}$ and $X_2=20$ min) in the DPPH scavenging effect (IC₅₀; 2.011 mg/mL) (Table 2-1) was near the surface center. Thus, the DPPH scavenging ability (IC₅₀) was lowered when the roasting temperature and time reached 110°C and 20 min, respectively.

ABTS radical scavenging effect Generation of the ABTS [2,2,9-

azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation (28) forms the basis of one of the spectrophotometric methods applied to measure the total antioxidant activity of solutions of pure substances (29), aqueous mixtures, and beverages (30). With regard to the ABTS radical scavenging effect (IC₅₀), we observed that the quadratic terms for temperature (X_1^2) had the largest effects (Table 4-1) with a high regression coefficient ($R^2=0.9697$, $p<0.0001$) (Table 3). The 3D response surface for ABTS radical scavenging activity (IC₅₀) is shown in Fig. 1B. The temperature greatly affected the ABTS scavenging activity (IC₅₀) and the roasting condition ($X_1=110^\circ\text{C}$ and $X_2=20$ min) in the ABTS scavenging effect (IC₅₀; 1.701 mg/mL) (Table 2-1) was near the surface center. Thus, the ABTS scavenging effect (IC₅₀) was lowered when the roasting temperature reached 110°C .

TPC Phenolic compounds, or polyphenols, are some of the most abundant structures in plants. Antioxidant compounds are usually phenolic in nature and their properties originate from their proton loss, chelating activities, and radical dismutations. Therefore, determination of the quantity of phenolic compounds is very important in order to determine the antioxidant capacity of plant extracts (31). Protective effects of *yak-kong* against postmenopausal bone loss and symptoms are associated with oxidative stress (16, 17). Kim *et al.* (15) reported that the more the amounts of *yak-kong* in coffee bean and *yak-kong* mixture was increased, the more NFATc1 expression in RANKL induced RAW 264.7 cells was reduced by high amounts of isoflavones and polyphenols in *yak-kong* compared to coffee bean. With regard to the TPC, we observed that the quadratic terms for temperature (X_1^2) had the largest effects (Table 4-1) with a high regression coefficient ($R^2=0.9381$, $p<0.0004$) (Table 3). The 3D

Fig. 1. Response surface plot of DPPH scavenging activity (A), ABTS radical scavenging activity (B) total polyphenol contents (TPC) (C), oxygen radical absorbance capacity (ORAC) value (D), 4 variables of anti-oxidation (DPPH scavenging activity, ABTS radical scavenging activity, TPC, and ORAC value) (E), nitric acid (NO) (F), prostaglandin E2 (PGE2) (G), tumor necrosis factor- α (TNF- α) (H), 3 variables of anti-inflammation (NO, PGE2, and TNF- α) (I), 7 variables of anti-oxidation and anti-inflammation (J) with roasting conditions for yak-kong.

response surface for TPC is presented in Fig. 1C. The roasting condition ($X_1=110^\circ\text{C}$ and $X_2=20$ min) for the TPC (52.573 mg TAE/g) (Table 2-1) was near the surface center. Thus, the TPC increased when the roasting temperature reached 110°C . This variable was optimized to achieve high total polyphenol content, i.e., more polyphenolic compounds indicated a higher antioxidant capacity for the extract.

ORAC value The ORAC value is widely used in academia and the food and supplement industries to determine the antioxidant capacity of a sample (32). The ORAC value detects chemical changes in a fluorescent molecule caused by a free radical attack and is based on peroxy radicals that reflect related physiological disturbances in a system (33). We observed that the quadratic terms for temperature (X_1 , X_1^2) had the largest effects (Table 4-1), which regression coefficient ($R^2=0.9823$, $p<0.0001$) was high (Table 3) concerning the ABTS radical scavenging activity (IC_{50}). The 3D response surface for ORAC is presented in Fig. 1C. The roasting condition ($X_1=110^\circ\text{C}$, $X_2=20$ min) in the ORAC ($7.097 \mu\text{mol TE/g}$) (Table 2-1) was near the surface center. Thus, the ORAC was increased when the roasting temperature reached 110°C .

NO production NO is associated with various biological process of inflammation (34,35). Therefore, the suppression of NO production by natural products may have potential therapeutic effect when connected with inflammation. Moreover, the suppressive effect of genistein shows mild suppressive effect against NO production from LPS-stimulated RAW 264.7 cells (36). With regard to the NO production, we observed that the quadratic terms for temperature (X_1^2) had the largest effects (Table 4-2) with a high regression coefficient ($R^2=0.9484$, $p<0.0002$) (Table 3). The 3D response surface for NO is shown in Fig. 1E. The temperature greatly affected NO production and the roasting condition ($X_1=110^\circ\text{C}$ and $X_2=20$ min) for NO production ($13.474 \mu\text{M}$) (Table 2-1) was near the surface center. Thus, the NO production decreased when the roasting temperature reached 110°C .

PGE2 production Overexpression of PGE2 is related to inflammation (37). With regard to the PGE2 production, we observed that the quadratic terms for temperature (X_1^2) had the largest effects (Table 4-2) with a high regression coefficient ($R^2=0.9647$, $p<0.0001$) (Table 3). The 3D response surface for PGE2 is presented in Fig. 1F. The temperature greatly affected PGE2 production and the roasting condition ($X_1=110^\circ\text{C}$ and $X_2=20$ min) for PGE2 production (3.341 mg/mL) (Table 2-1) was near the surface center. Thus, the PGE2 was lowered when the roasting temperature reached 110°C .

TNF- α Tumor necrosis factor α (TNF- α) is a pro-inflammatory cytokine that is clearly associated with the pathogenesis of inflammatory diseases (38). We observed that the quadratic terms for temperature (X_1^2) had the largest effects (Table 4-2), which

Table 5. Predicted values of optimum roasting condition for response variables

Response variables	Optimum roasting condition		Predicted values
	Temperature	Time	
Y_1 (DPPH)	111.09	19.90	2.141
Y_2 (ABTS)	112.46	19.42	1.770
Y_3 (TPC)	109.52	21.07	51.458
Y_4 (ORAC)	112.70	21.87	6.9695
Y_5 (NO)	111.11	20.30	14.450
Y_6 (PGE2)	110.53	20.44	3.430
Y_7 (TNF- α)	109.10	22.67	3.810

regression ($R^2=0.9057$, $p<0.0001$) was high coefficient as to the TNF- α production (Table 3). The 3D response surface for TNF- α is presented in Fig. 1G. The temperature greatly affected the TNF- α production and the roasting condition ($X_1=110^\circ\text{C}$, $X_2=20$ min) for TNF- α production (3.713 mg/mL) (Table 2-1) was near the surface center. Thus, the TNF- α was lowered when roasting temperature reached 110°C .

Verification of predictive models The predicted values of *yak-kong* and roasting conditions are presented in Table 5. The minimum DPPH IC_{50} value of *yak-kong* was observed at the roasting conditions of 111.09°C for 19.90 min (Table 5, Fig. 1A), and the predicted DPPH IC_{50} was 2.141 mg/mL . The minimum ABTS IC_{50} value of *yak-kong* was observed under the roasting conditions of 112.46°C for 19.42 min (Table 5, Fig. 1B), and the predicted ABTS IC_{50} was 1.770 mg/mL . The maximum TPC value of *yak-kong* was at the roasting conditions of 109.52°C for 21.07 min (Table 5, Fig. 1C), with a predicted TPC value of 51.458 TAE/g . The maximum ORAC value of *yak-kong* was with roasting at 112.70°C for 21.87 min (Table 5, Fig. 1D), and the predicted TPC value was $6.970 \mu\text{mol TE/g}$. Optimum roasting conditions (temperature and time) with regard to the 4 variables associated with anti-oxidation were 111.47°C for 20.45 min (Fig. 1E), resulting in predicted values of 2.143, 1.775 mg/mL , and 51.389 mg TAE/g $6.893 \mu\text{mol TE/g}$ for DPPH IC_{50} , ABTS IC_{50} , TPC, and ORAC, respectively. The minimum NO production of *yak-kong* was at the roasting conditions of 111.11°C for 20.30 min (Table 5, Fig. 1F), and the predicted NO production was $14.450 \mu\text{M}$. The minimum PGE2 production of *yak-kong* occurred under the roasting conditions of 110.53°C for 20.44 min (Table 5, Fig. 1G), and the predicted PGE2 production was 3.430 mg/mL . The minimum TNF- α of *yak-kong* was observed at the roasting conditions of 109.10°C for 22.67 min (Fig. 1H), with a predicted TNF- α value of 3.810 ng/mL . Optimum roasting conditions (temperature and time) as to the 3 variables related to anti-inflammation were 110.24°C for 21.18 min (Fig. 1I), which resulted in predicted values of $14.484 \mu\text{M}$, 3.433 mg/mL , and 3.818 ng/mL for NO, PGE2, and TNF- α , respectively.

Optimum roasting conditions (temperature and time) with regard to the seven variables related to anti-oxidation and anti-inflammation were 110.88°C for 20.86 min (Fig. 1J), which resulted in predicted

values of 2.143, 1.781 mg/mL, 51.419 mg TAE/g, 6.893 μ mol TE/g, 14.460 μ M, 3.432 mg/mL, and 3.823 ng/mL for DPPH (IC_{50}), ABTS (IC_{50}), TPC, ORAC, NO, PGE2, and TNF- α , respectively. Kim *et al.* (15) reported that the one to one mixture of coffee bean and yak-kong (Cb50RoS50) is useful as a coffee alternative beverage because the sensory evaluation was similar to that of coffee bean alone (Cb100RoS0).

It was concluded that RSM was performed to determine the optimal roasting conditions for yak-kong for use as a health beverage for bone health instead of coffee beans, which promoted increased calcium extraction, inhibition of osteoblast proliferation, and increased osteoclast proliferation (39). The optimum roasting conditions (temperature and time) with regard to the seven variables related to anti-oxidation and anti-inflammation were 110.88°C for 20.86 min.

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