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Full Length Research Paper

Optimum extraction conditions for arbutin from Asian pear peel by supercritical fluid extraction (SFE) using Box-Behnken design

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Asian pear (*Pyrus pyrifolia* cv. Niitaka) peel, a by-product that results from juice processing, is a good source of arbutin, a polyphenol, which has a whitening effect on the skin. The objective of this study was to investigate the optimum extraction conditions for arbutin from Asian pear peel by supercritical fluid extraction (SFE) using response surface methodology (RSM) based on Box-Behnken experiment design. Arbutin was extracted using co-solvent, methanol and ethanol. A three-level four-factor Box-Behnken experiment design was performed to evaluate the combination effect of four independent variances, co-solvent concentration (22 to 30%), extraction pressure (250 to 300 bar), extraction temperature (30 to 60°C) and extraction time (30 to 60 min), coded for X_1, X_2, X_3 and X_4, respectively. The coefficients of determination (R^2) of response surface regression equations were 0.89 ($p<0.01$) for methanol as a co-solvent and 0.84 ($p<0.01$) for ethanol. Arbutin content by SFE with methanol was the highest with 3.35 mg/g at 26%, 275 bar, 45°C and 45 min. In conclusion, arbutin from Asian pear peel would be extracted from the most efficiently combination of 26% methanol as co-solvent, extraction pressure of 275 bar, extraction temperature of 45°C and extraction time of 45 min in the SFE.

Key words: Asian pear, arbutin, response surface methodology, supercritical fluid extraction, Box-Behnken experiment design.

INTRODUCTION

Pear (*Pyrus*) species originates from the western mountainous area of China and includes the oriental pear and occidental pear and they are distributed mainly around Eastern Asia, including China, Korea and Japan; the major cultivated species include *Pyrus bretschnrideri* Reh., *P. pyrifolia* Nakai, *Pyrus ussuriensis* Maxim and *Pyrus sinkiangensis* Yu (Cui et al., 2005). The occidental pear contains phenolic compounds, such as chlorogenis acid, rutin, procuanidins and arbuin. These phenolic compounds were investigated for their activity as antioxidants or as coloring factors in the fruit and their products. Arbutin, another important phenolic compound in pear fruit, was initially identified as an antibiotic substance in fire blight resistance and later as a specific marker or pear products for the evaluation of product authenticity (Frias et al., 2006). Arbutin (hydroquinone-β-D-glucopyranoside) is a natural phenolic glucoside found in various plant species of diverse families, such as Ericaceae (*Vaccinium* spp., *Arctostaphylos* spp.), Asteraceae (*Achillea millefolium*), Betulaceae (*Betula alba*) and Rosaceae (*Pyrus communis* L.). Arbutin is commonly used in urinary therapeutics (Zhai and Maibach, 2001) and as a human skin-whitening agent (Tomita, 1990). This latter action was attributed mainly to its inhibitory effect on melanosomal tyrosinase activity, rather than suppression of the expression and synthesis of tyrosinase (Jin, 1999). Arbutin is found in extremely high concentrations in certain resurrection plants (Maeda

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Abbreviations: SFE, Supercritical fluid extraction; **RSM,** response surface methodology; **PLA2,** phospholipase A2; **L-DOPA,** L-3, 4-dihydroxyphenylalanine; **HPLC,** High-performance liquid chromatography; **RSREG,** response surface regression; **SAS,** statistical analysis system.

Table 1. Levels of independent variables for Box-Behnken experiment design in extraction of arbutin.

Independent variable	Levels		
	-1	0	1
Co-solvent concentration (%, X1)	22	26	30
Extraction pressure (bar, X2)	250	275	300
Extraction Temp (°C, X3)	32	45	60
Extraction Time (min, X4)	90	120	150

and Fukuda, 1991; Maeda and Fukuda, 1996) and as the species that accumulate it survive extreme environmental stresses, such as frost and drought, arbutin may contribute to their stress hardiness.

The physiological role of arbutin in resurrection plants is unknown, but it is believed that arbutin contributes to the protection of membrane components in the dry state (Escarpa and Gonzalez, 1999), as it has been shown to be an antioxidant (Couteau and Coiffard, 2001) and also to inhibit phospholipase A2 (PLA2) activity in mostly dehydrated systems (Masse, 2001). Arbutin is a solute accumulated to high concentration in drought and frost resistant plant. This hydroquinone derivative composed by glucose and a phenol moiety is isolated from the leaves of the bearberry shrub, cranberry, blueberry and most types of pears (Frias et al., 2006). Arbutin is a skin care products and as a whitening agent, it can compete with L-DOPA for receptor site on tyrosinase and hinders the oxidation of L-DOPA, thrums can inhibit the formation of eumelanin (Lin et al., 2007).

Unfortunately, few studies deal with method development and validation using statistical designs and response surface techniques to determine the optimum operational conditions for the hydrolysis. The conventional approach for the optimization of a multivariable system is usually one variable at a time. This can be very time-consuming and when interactions exist between the variables, it is unlikely to find the true optimum. RSM is a very useful tool for this purpose as it provides statistical models that help in understanding the interactions among the parameters that should be optimized.

This aim of this study was to investigate the optimum extraction conditions for arbutin from Asian pear peel by SFE using RSM based on Box-Behnken experimental design.

MATERIALS AND METHODS

Asian pear cultivars (*P. pyrifolia* cv. Niitaka), is grown in private orchard in Naju city of South Korea was used for this study. In 2007 harvest season, the fruits were harvested carefully by hand at their commercial maturity stage and transferred to the laboratory.

Supercritical fluid extraction (SFE) condition

Extractions were carried out for SFE (Insong) with a 100 g extraction cell. The extraction pressure was controlled by micro metering valves and the carbon dioxide pump was from Bran-Luebbe (Norderstedt, Germany). Fractionation was achieved in two different vessels, with independent temperature and pressure control, by a decrease in pressure.

The extraction cell was filled up with 60 g of ground laurel and 90 g of washed sea sand (Panreac, Barcelona, Spain). Dynamic extraction was performed at the following experimental conditions: extraction pressure, 250 bars; extraction temperature, 60°C; 4% of ethanol as modifier; pressure of separator 1, 100 bar; temperature of separator 1, 60°C; pressure of separator 2, 20 bar and temperature of separator 2, 20°C. Extraction time was 75 min and the addition of ethanol started when selected pressure was reached. All extracts were kept under N2, at 20°C in the dark and ethanol was eliminated at 35°C in a vacuum rotary evaporator.

Experimental design

Optimization of conditions for arbuitn from Asian pear was carried out using RSM. Experiments with four independent variables, co-solvent concentration (X1), extraction pressure (X2), extraction temperature (X3) and extraction time (X4) were conducted following the experimental design statistical analysis obtained by the Box–Behnken experimental design.

This design was selected due to the small number of experiments required to estimate complex response functions. For the three-level four factorial Box–Behnken experimental were design, a total of 27 experimental that were runs are necessary. The uncoded and coded independent variables and experimental design are listed in Tables 1 and 2.

Determination of arbutin

The arbutin (4-hydroxyphenyl-â-D-glucopyranoside) contents of samples were determined by HPLC. The column used was waters spherisorb ODS2 (25.0 × 0.46 cm, 5 µm). The mobile phase was water/formic acid (19:1, v/v) and methanol at a flow rate of 0.9 mL/min. Eluates were detected at 280 nm (UV-975, Jasco, Japan).

Statistical analysis

The experimental data (Table 1) were analyzed by response surface regression (RSREG) procedures using SAS software to fit the following second-order polynomial Equation (1):

$$Y = b_0 + \sum_{i=1}^{4} b_i x_i + \sum_{i=1}^{4} b_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} b_{ij} x_i x_j \quad (1)$$

Where Y is the response (percent of molar conversion); b_0 is a constant, b_i, b_{ii} and b_{ij} are coefficients; x_i and x_j are the uncoded

Table 2. Box-Behnken experiment design setting in the original and coded form of the independent variables (X_1, X_2, X_3 and X_4).

No	X_1	X_2	X_3	X_4	Co-solvent concentration (%)	Extraction pressure (bar)	Extraction temp (℃)	Extraction time (min)
1	-1	-1	0	0	22	250	45	120
2	0	-1	-1	0	26	250	32	120
3	0	-1	0	-1	26	250	45	90
4	0	-1	0	1	26	250	45	150
5	0	-1	1	0	26	250	60	120
6	1	-1	0	0	30	250	45	120
7	-1	0	-1	0	22	275	32	120
8	-1	0	0	-1	22	275	45	90
9	-1	0	0	1	22	275	45	150
10	-1	0	1	0	22	275	60	120
11	0	0	-1	-1	26	275	32	90
12	0	0	-1	1	26	275	32	150
13	0	0	0	0	26	275	45	120
14	0	0	0	0	26	275	45	120
15	0	0	0	0	26	275	45	120
16	0	0	1	-1	26	275	60	90
17	0	0	1	1	26	275	60	150
18	1	0	-1	0	30	275	32	120
19	1	0	0	-1	30	275	45	90
20	1	0	0	1	30	275	45	150
21	1	0	1	0	30	275	60	120
22	-1	1	0	0	22	300	45	120
23	0	1	-1	0	26	300	32	120
24	0	1	0	-1	26	300	45	90
25	0	1	0	1	26	300	45	150
26	0	1	1	0	26	300	60	120
27	1	1	0	0	30	300	45	120

independent variables. The options of RSREG SAS and RIDGE MAX were employed to compute the estimated ridge of maximum response for increasing radii from the center of the original design.

RESULTS AND DISCUSSION

Optimization of extraction conditions using methanol

Figure 1 shows the three dimensional plots of the effect of the independent variables co-solvent concentration and extraction pressure on the arbutin content with SFE extraction. Response surface for the effect of extraction temperature and co-solvent concentration on arbutin content of arbutin extracted from Asian pear peel (Figure 2). Three dimensional plots of the effect of the independent variables extraction time and co-solvent concentration on the arbutin content with SFE extraction (Figure 3). Figure 4 shows the three dimensional plots of the effect of the independent variables of extraction temperature and extraction pressure on the arbutin content with SFE extraction.

Response surface for the effect of extraction time and extraction pressure on arbutin content of arbutin extracted from Asian pear peel (Figure 5). Three dimensional plots of the effect of the independent variables extraction time and extraction temperature on the arbutin content with SFE extraction (Figure 6). In order to obtain a model for CAPE synthesis, the results from the 3-level-4-factor Box-Behnken design (Table 3) were used and the RSREG procedure from SAS was employed to fit the second-order polynomial Equations 1 and 2 was thus generated and is given as:

$$Y_{MeOH} = 31.695451 + 0.442257X_1 + 0.185411X_2 + 0.105694X_3 + 0.061407X_4 - 0.007786X_1^2 + 0.000083X_2X_1 - 0.000343X_2^2 - 0.000764X_3X_1 - 0.000044X_3X_2 - 0.000815X_3^2 - 0.000528X_4X_1 + 0.000067X_4X_2 - 0.000026X_4X_3 - 0.000717X_4^2 \quad (R^2=0.89) \qquad (2)$$

Analysis of variance indicates that this second-order polynomial model was highly significant and adequate to represent the actual relationship between the response (percent molar conversion) and the variables. The p-value

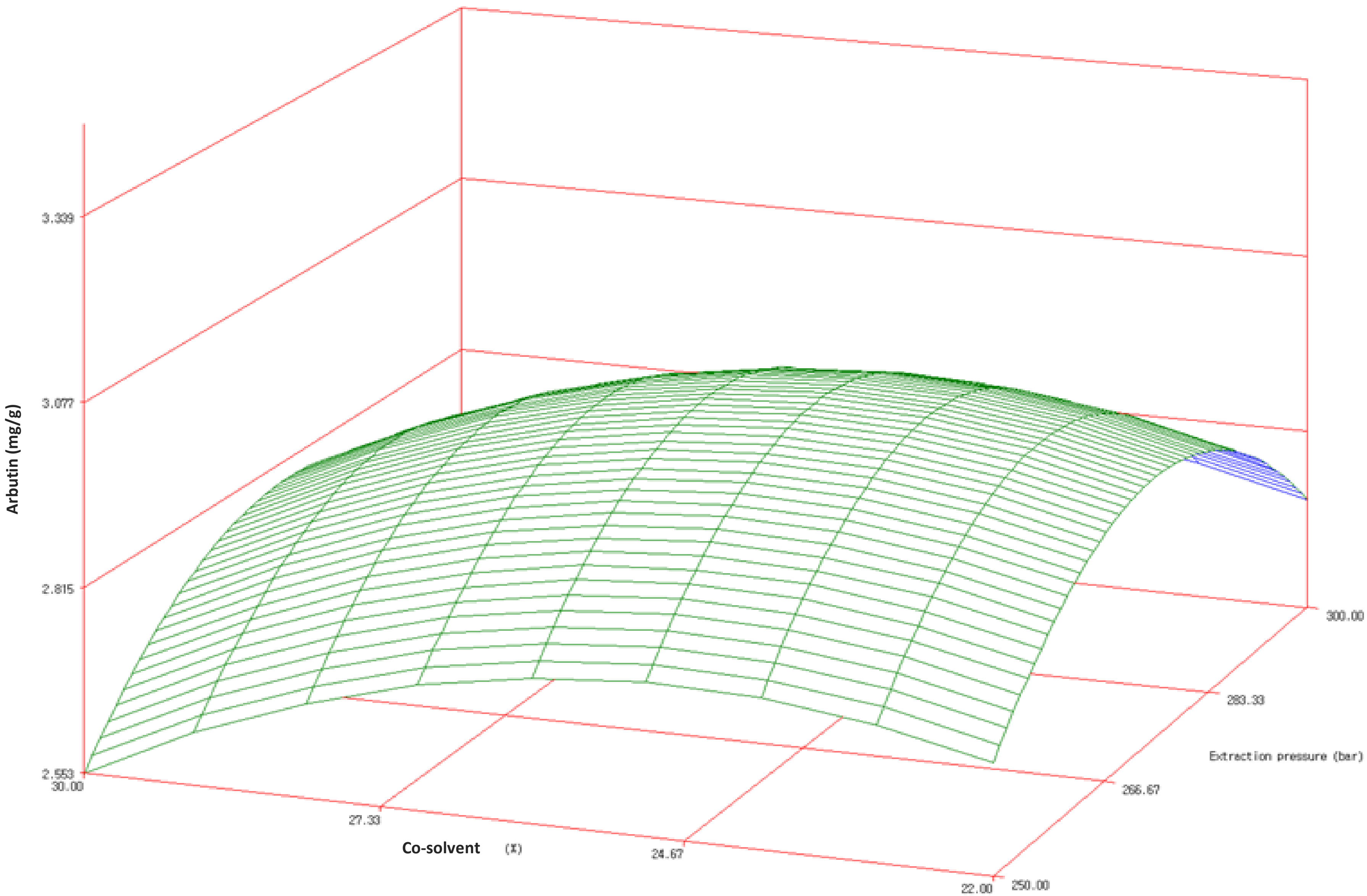

Figure 1. Response surface for the effect of extraction pressure and co-solvent concentration on arbutin content of arbutin extracted from Asian pear peel.

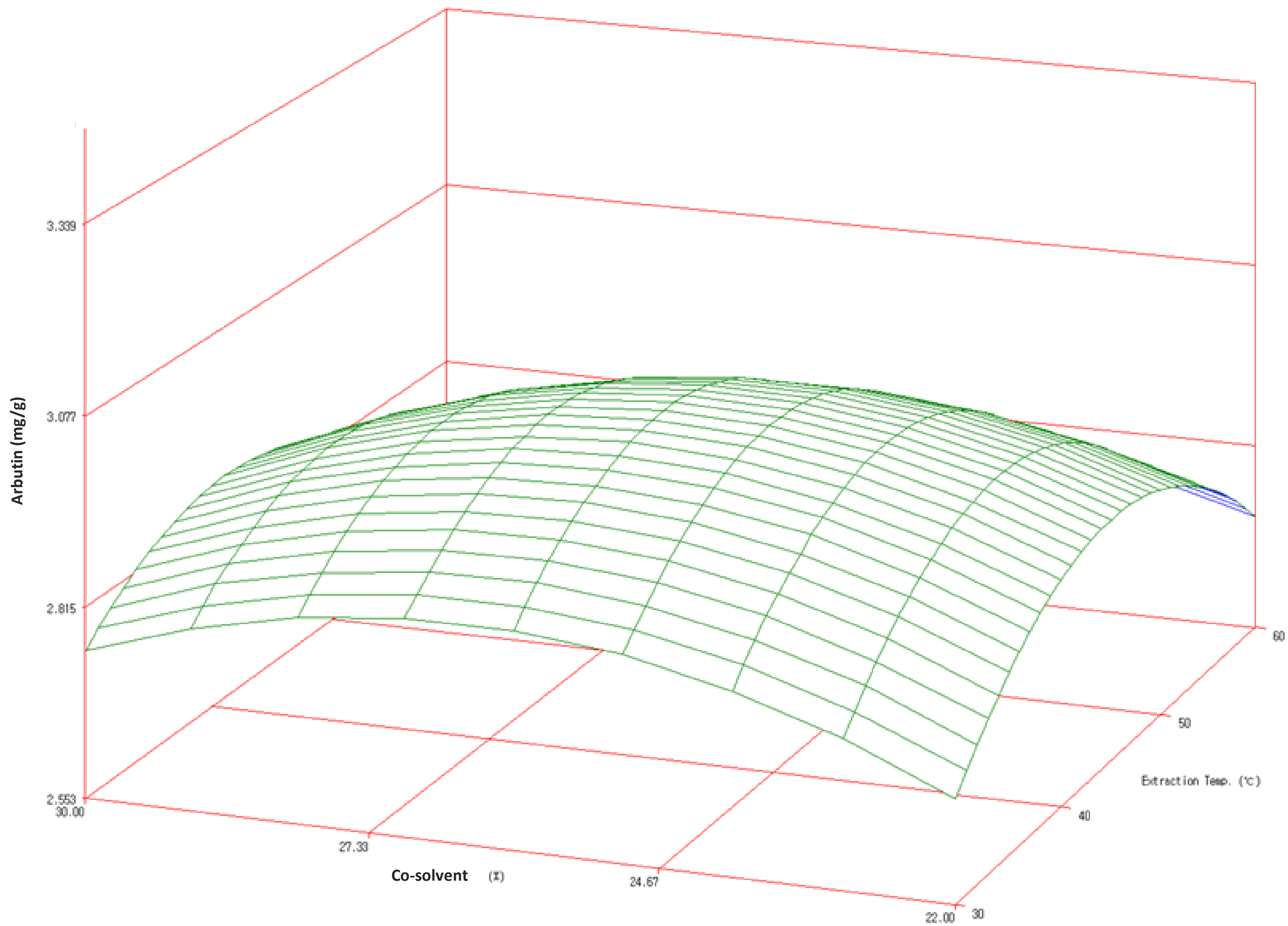

Figure 2. Response surface for the effect of extraction temperature and co-solvent concentration on arbutin content of arbutin extracted from Asian pear peel.

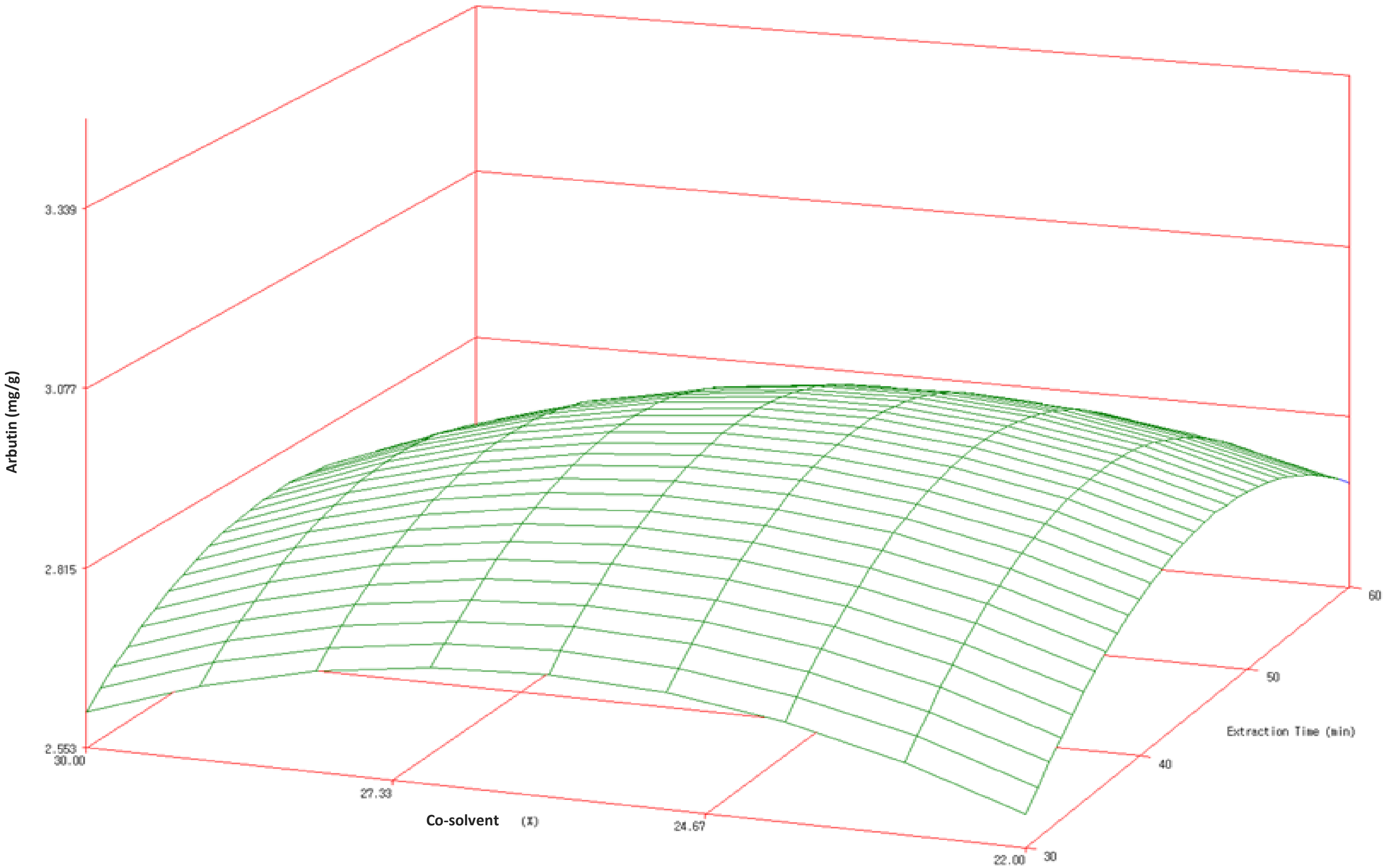

Figure 3. Response surface for the effect of extraction time and co-solvent concentration on arbutin content of arbutin extracted from Asian pear peel.

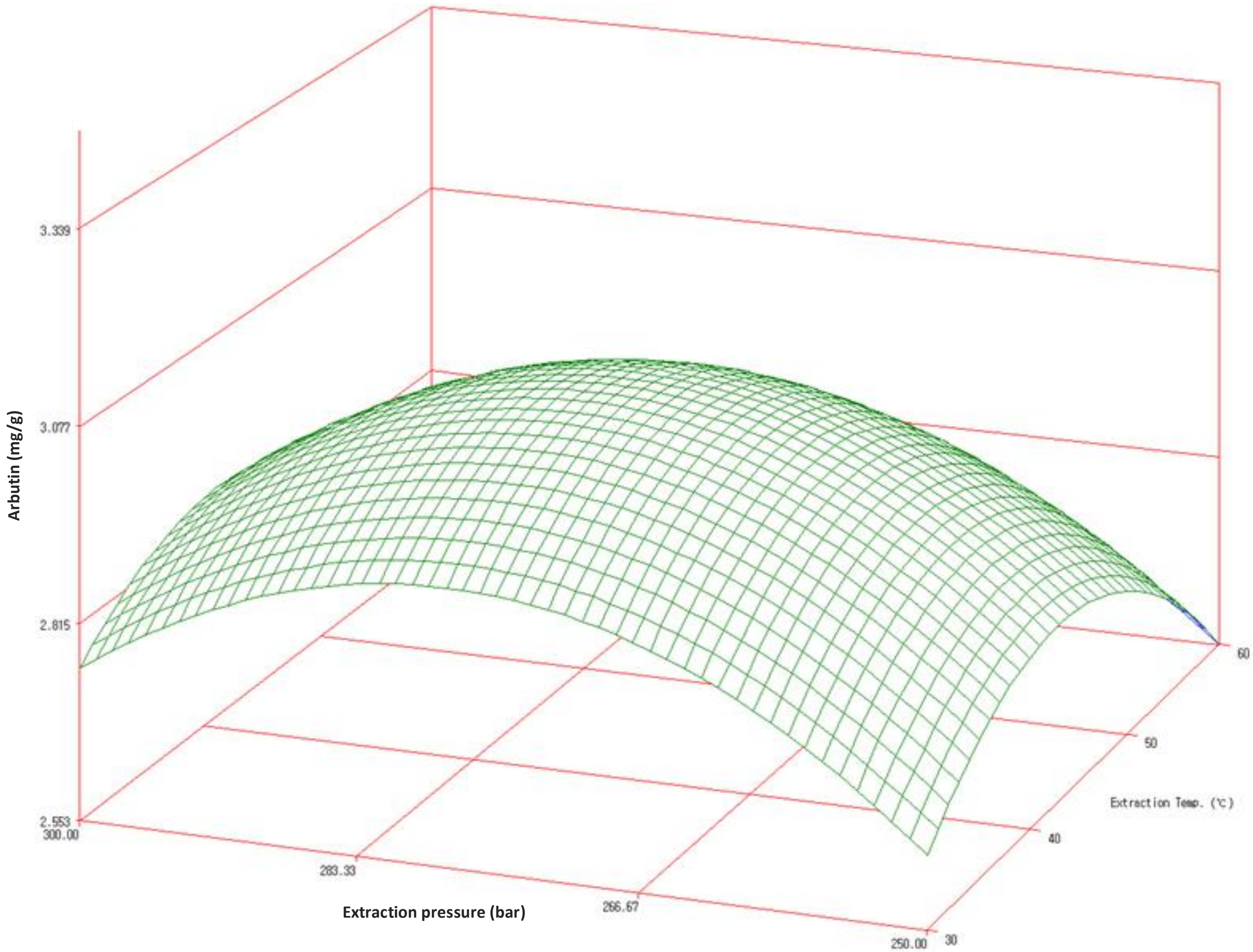

Figure 4. Response surface for the effect of extraction temperature and extraction pressure on arbutin content of arbutin extracted from Asian pear peel.

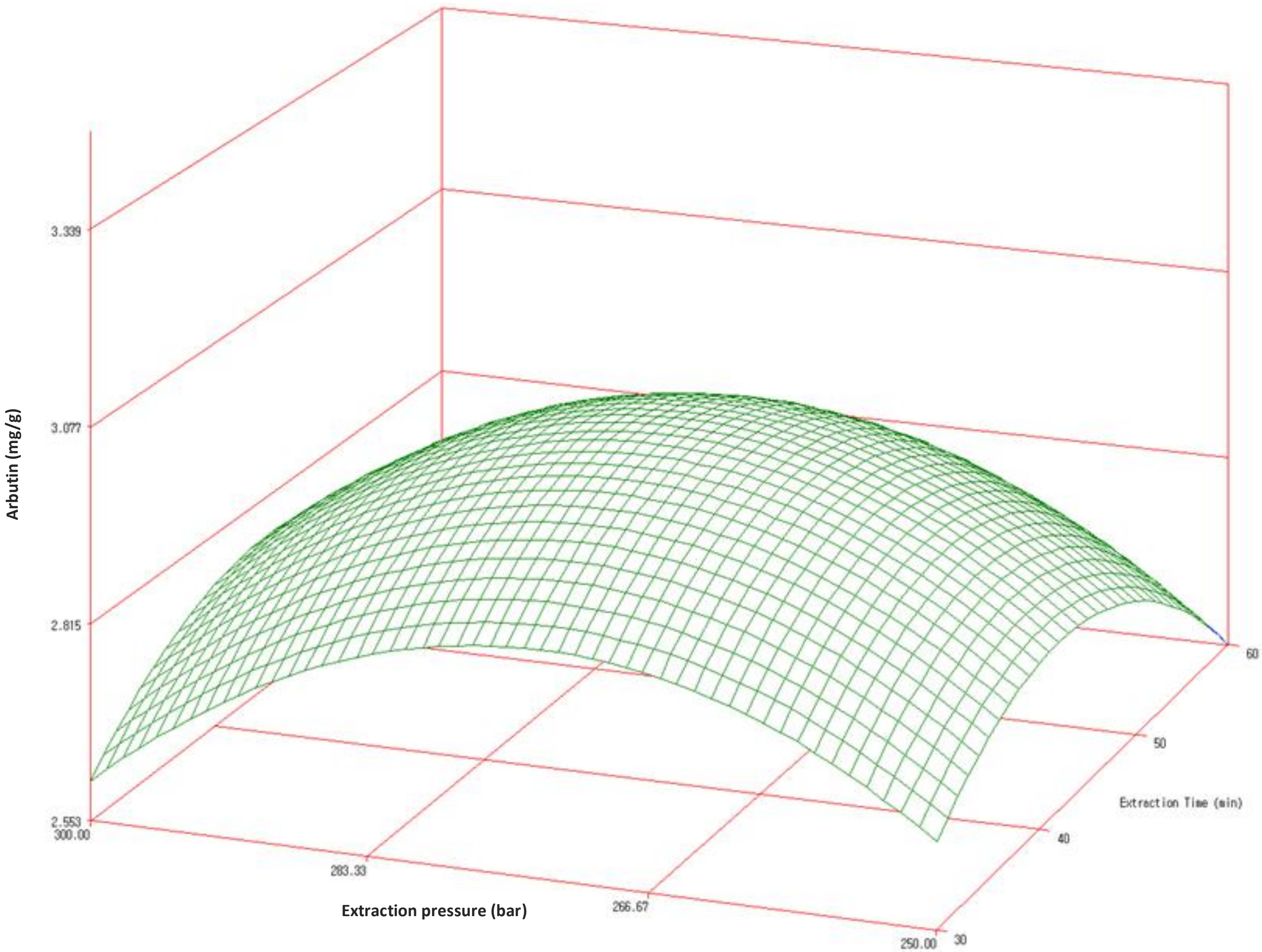

Figure 5. Response surface for the effect of extraction time and extraction pressure on arbutin content of arbutin extracted from Asian pear peel.

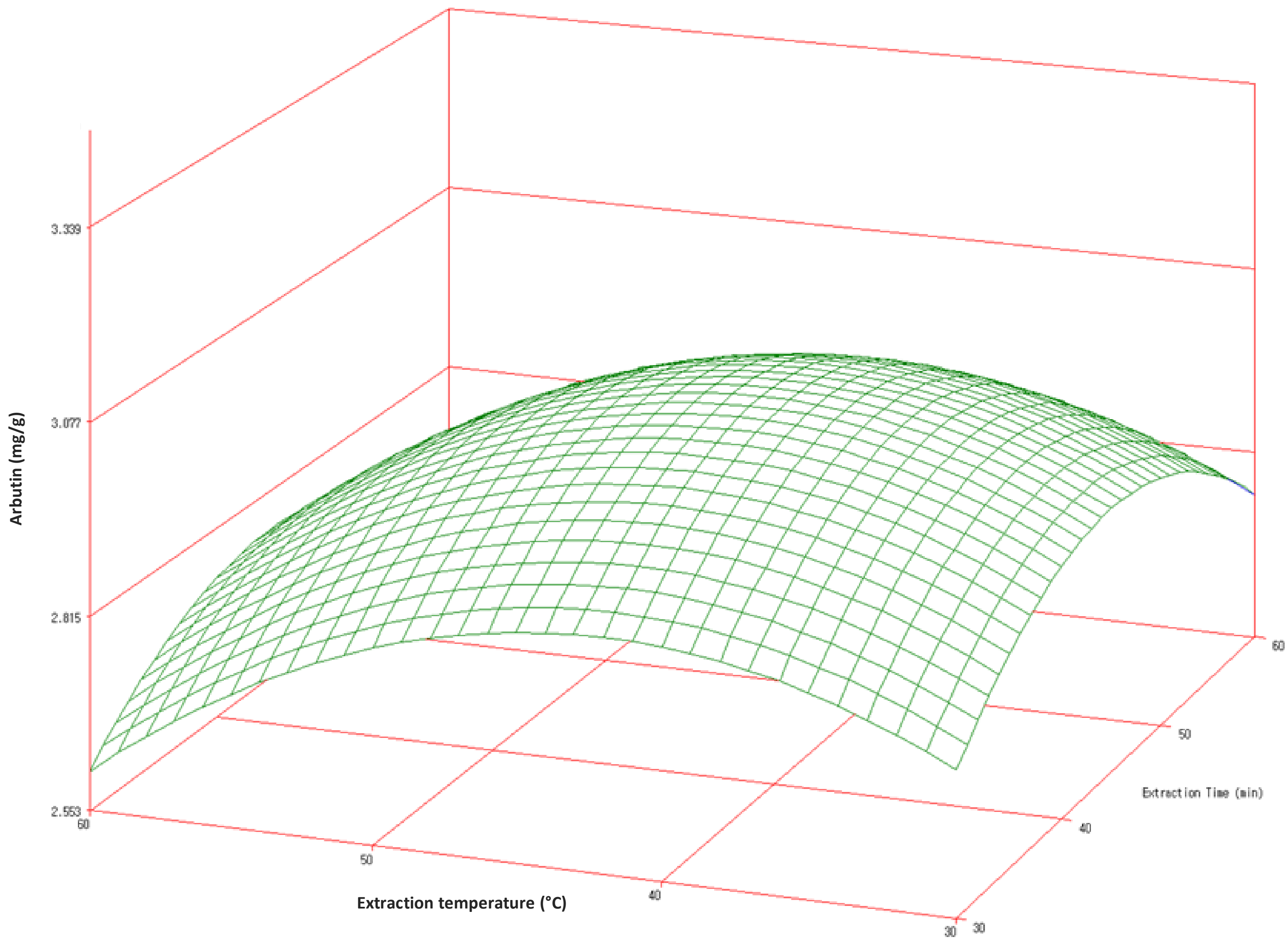

Figure 6. Response surface for the effect of extraction time and extraction temperature on arbutin content of arbutin extracted from Asian pear peel.

Table 3. Arbuitn content of Asian pear peel after extracting with supercritical fluid (unit: mg/g).

No	Co-solvent concentration (%)	Extraction pressure (bar)	Extraction temp (℃)	Extraction time (min)	Arbutin content (mg/g)	
					MeOH	EtOH
1	22	250	45	120	3.22 ±0.01	2.45 ±0.01
2	26	250	32	120	3.18 ±0.02	2.53 ±0.02
3	26	250	45	90	3.28 ±0.02	2.45 ±0.01
4	26	250	45	150	3.27 ±0.03	2.57 ±0.01
5	26	250	60	120	3.18 ±0.02	2.46 ±0.01
6	30	250	45	120	3.34 ±0.03	2.56 ±0.03
7	22	275	32	120	3.13 ±0.02	2.44 ±0.01
8	22	275	45	90	3.27 ±0.03	2.56 ±0.01
9	22	275	45	150	3.35 ±0.02	2.54 ±0.03
10	22	275	60	120	3.35 ±0.02	2.54 ±0.03
11	26	275	32	90	3.35 ±0.02	2.54 ±0.03
12	26	275	32	150	3.28 ±0.01	2.56 ±0.03
13	26	275	45	120	3.16 ±0.01	2.54 ±0.02
14	26	275	45	120	3.13 ±0.02	2.52 ±0.02
15	26	275	45	120	3.10 ±0.01	2.54 ±0.03
16	26	275	60	90	3.28 ±0.03	2.45 ±0.01
17	26	275	60	150	3.18 ±0.02	2.55 ±0.04
18	30	275	32	120	2.31 ±0.02	1.76 ±0.02
19	30	275	45	90	2.44 ±0.03	1.94 ±0.03
20	30	275	45	150	2.58 ±0.02	2.10 ±0.02
21	30	275	60	120	2.64 ±0.03	2.13 ±0.03
22	22	300	45	120	2.59 ±0.01	2.13 ±0.01
23	26	300	32	120	2.58 ±0.02	2.11 ±0.02
24	26	300	45	90	2.48 ±0.02	2.04 ±0.02
25	26	300	45	150	2.46 ±0.01	2.10 ±0.01
26	26	300	60	120	2.45 ±0.01	2.04 ±0.04
27	30	300	45	120	2.52 ±0.02	2.03 ±0.02

was <0.001 and the coefficient of determination (R^2) were 0.89. The variables with a significant effect on arbutin content were the co-solvent concentration (X_1), extraction pressure (X_2), extraction temperature (X_3) and extraction time (X_4) (*P <0.01*).

Optimization of extraction conditions using ethanol

Figure 7 shows the three dimensional plots of the effect of the independent variables co-solvent concentration and extraction pressure on the arbutin content with SFE extraction. Response surface for the effect of extraction temperature and co-solvent concentration on arbutin content of arbutin extracted from Asian pear peel (Figure 8).

Three dimensional plots of the effect of the independent variables extraction time and co-solvent concentration on the arbutin content with SFE extraction (Figure 9). Figure 10 shows the three dimensional plots of the effect of the independent variables of extraction temperature and extraction pressure on the arbutin content with SFE extraction. Response surface for the effect of extraction time and pressure on arbutin content of arbutin extracted from Asian pear peel (Figure 11). Three dimensional plots of the effect of the independent variables extraction time and temperature on the arbutin content with SFE extraction (Figure 12).

In order to obtain a model for CAPE synthesis, the results from the 3-level-4-factor Box-Behnken design (Table 3) were used and the RSREG procedure from SAS was employed to fit the second-order polynomial Equations (1and 3) was thus generated and is given as:

$$Y_{EtOH} = 25.536773 + 0.002742X_1 + 0.014999X_2 + 0.073192X_3 + 3.254124X_4 + 0.003046X_1^2 + 0.001827X_2X_1 - 0.016737X_2^2 - 0.004959X_3X_1 - 0.007839X_3X_2 - 0.052510X_3^2 + 0.0024851X_4X_1 + 0.0005173X_4X_2 + 0.000012X_4X_3 + 0.004835X_4^2 \ (R^2=0.84) \quad (3)$$

Analysis of variance indicates that this second-order polynomial model was highly significant and adequate to represent the actual relationship between the response (percent molar conversion) and the variables. The p-value

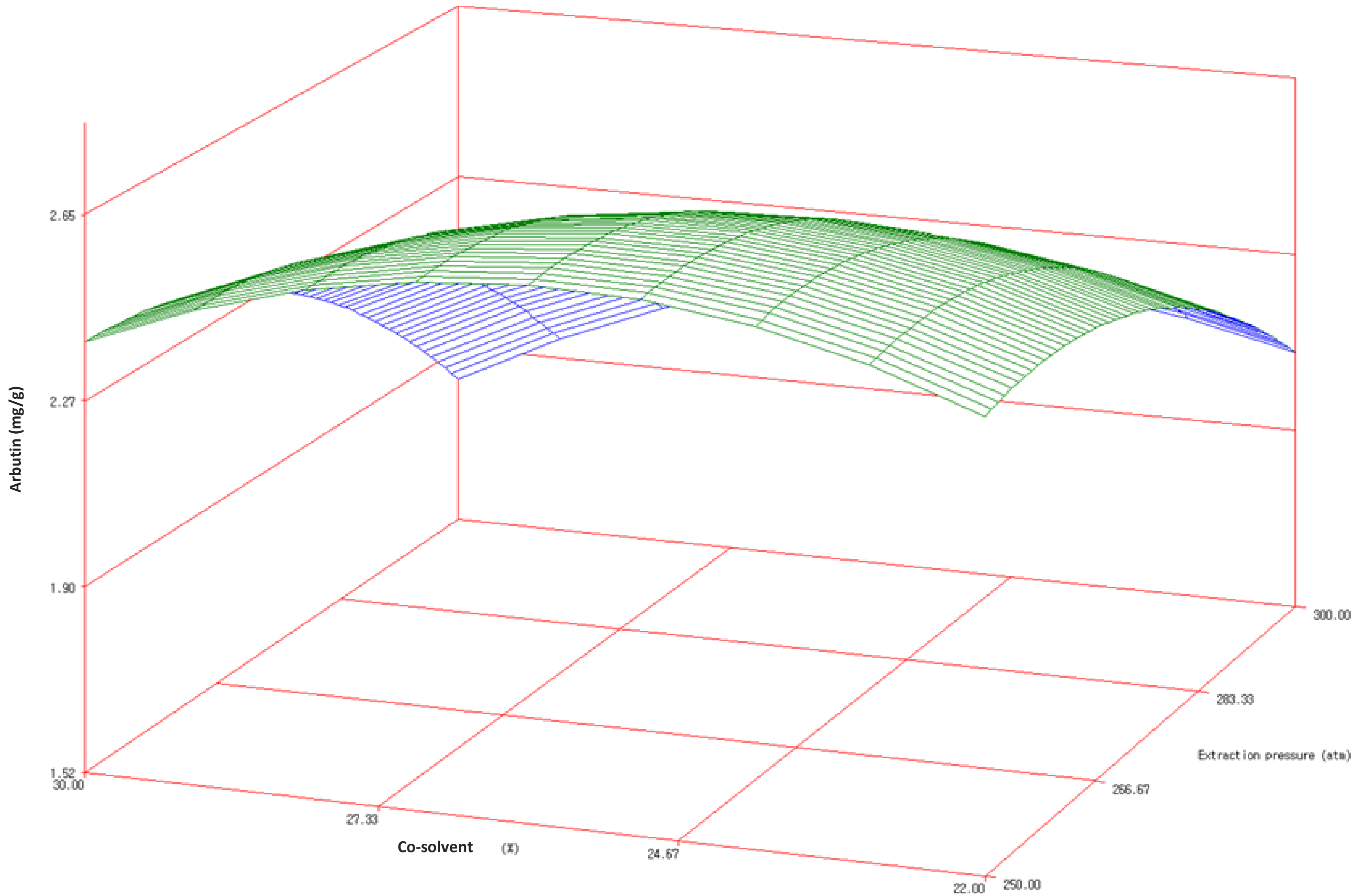

Figure 7. Response surface for the effect of extraction pressure and co-solvent concentration on arbutin content of arbutin extracted from Asian pear peel.

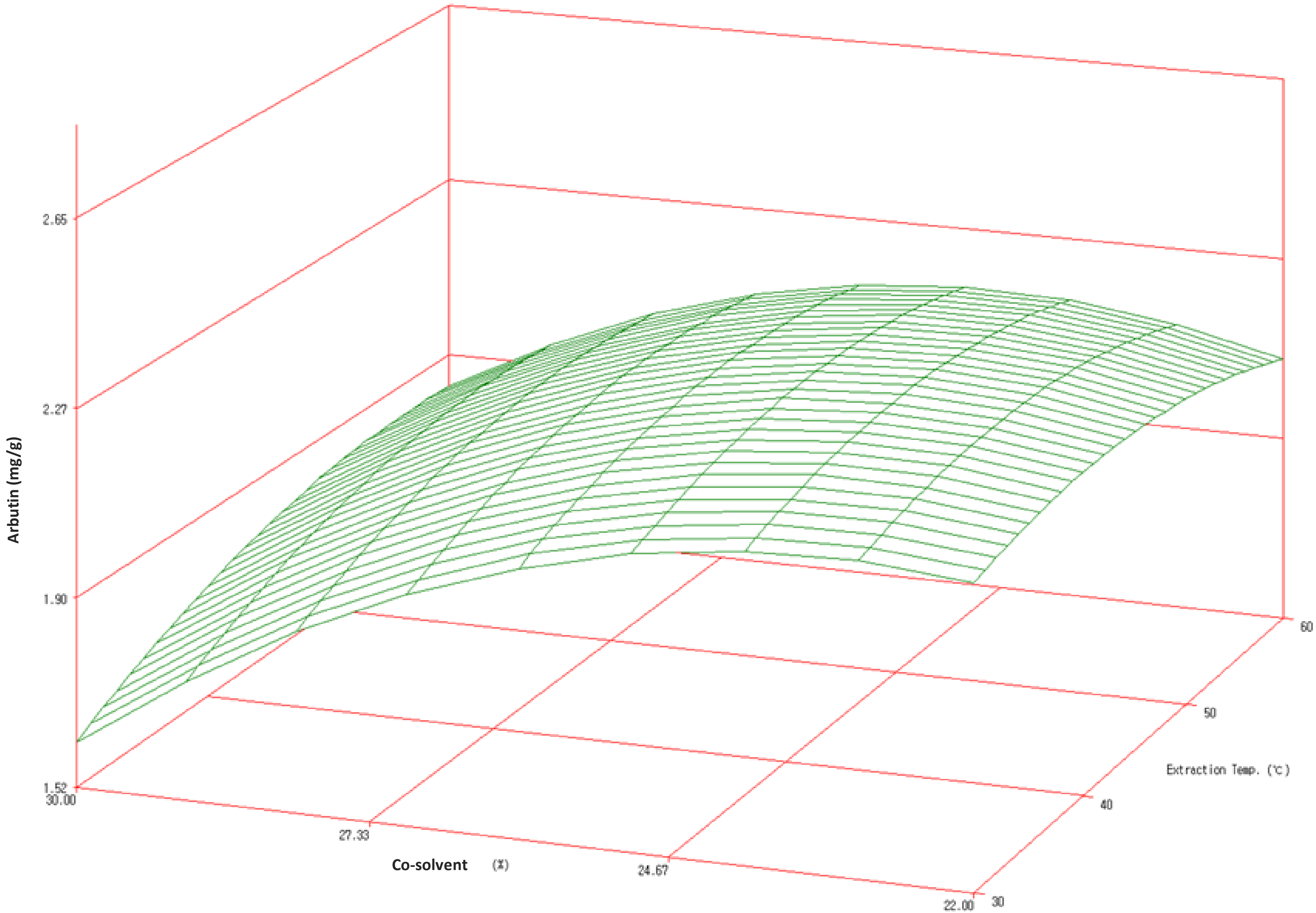

Figure 8. Response surface for the effect of extraction temperature and co-solvent concentration on arbutin content of arbutin extracted from Asian pear peel.

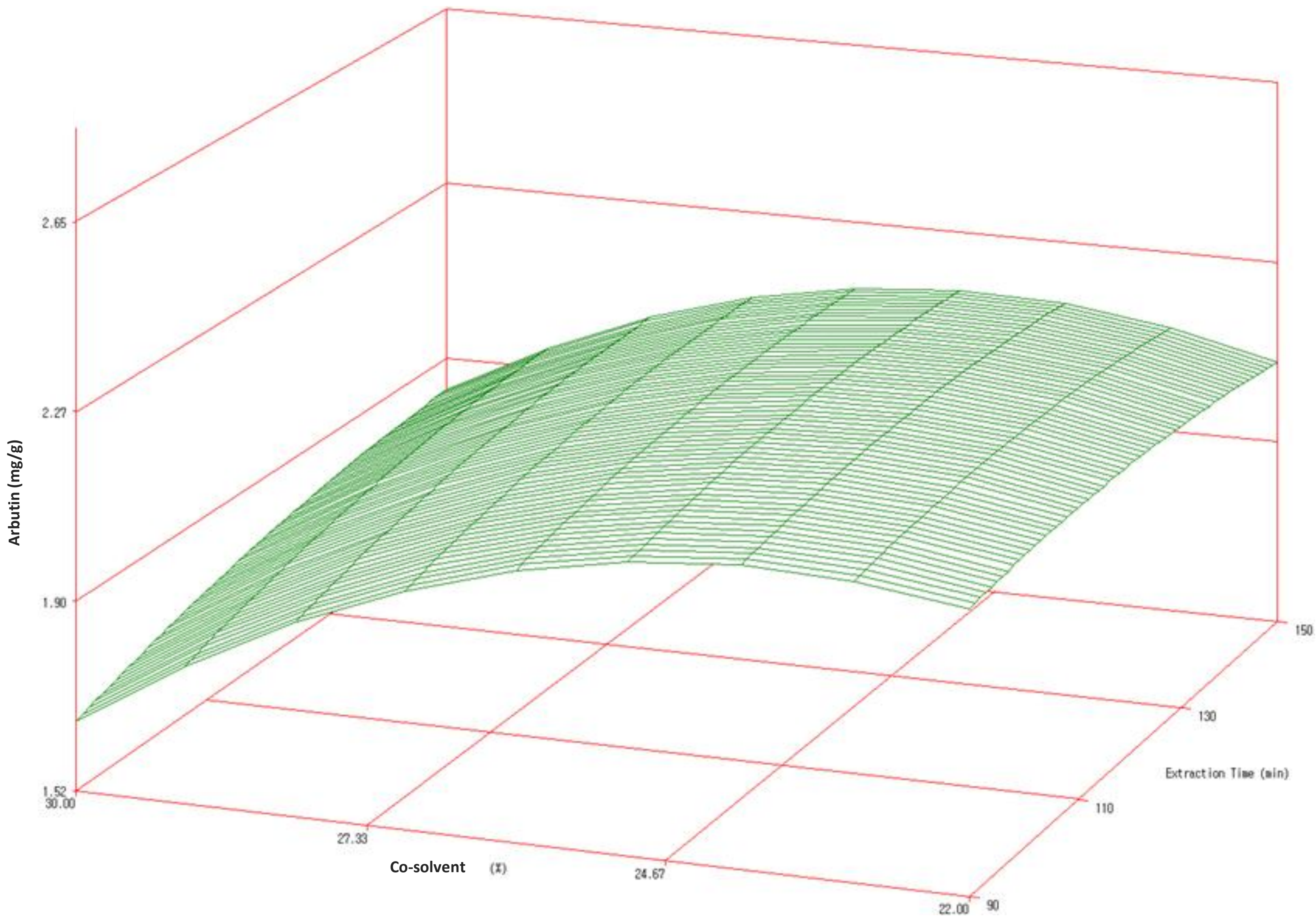

Figure 9. Response surface for the effect of extraction time and co-solvent concentration on arbutin content of arbutin extracted from Asian pear peel.

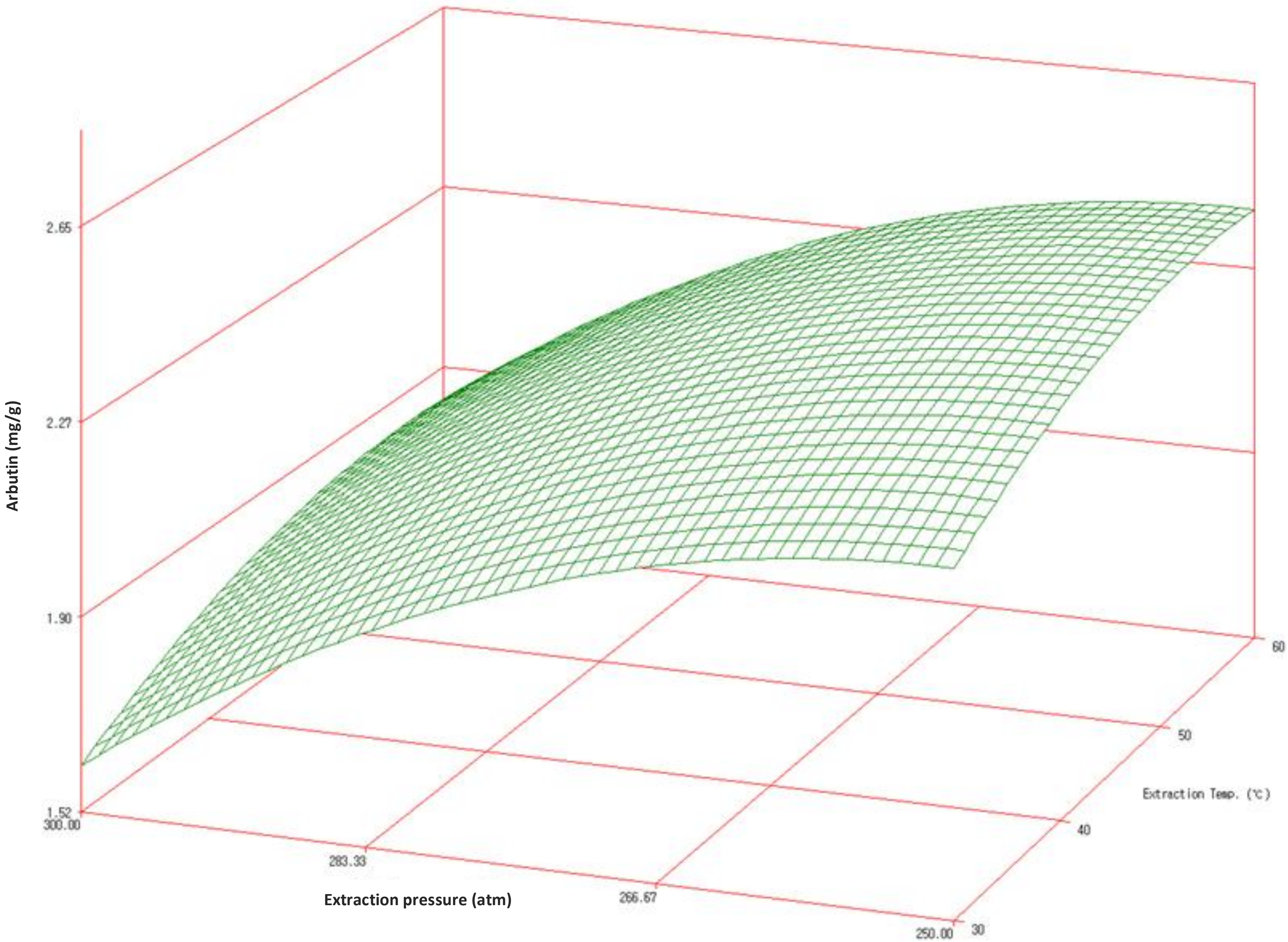

Figure 10. Response surface for the effect of extraction temperature and extraction pressure on arbutin content of arbutin extracted from Asian pear peel.

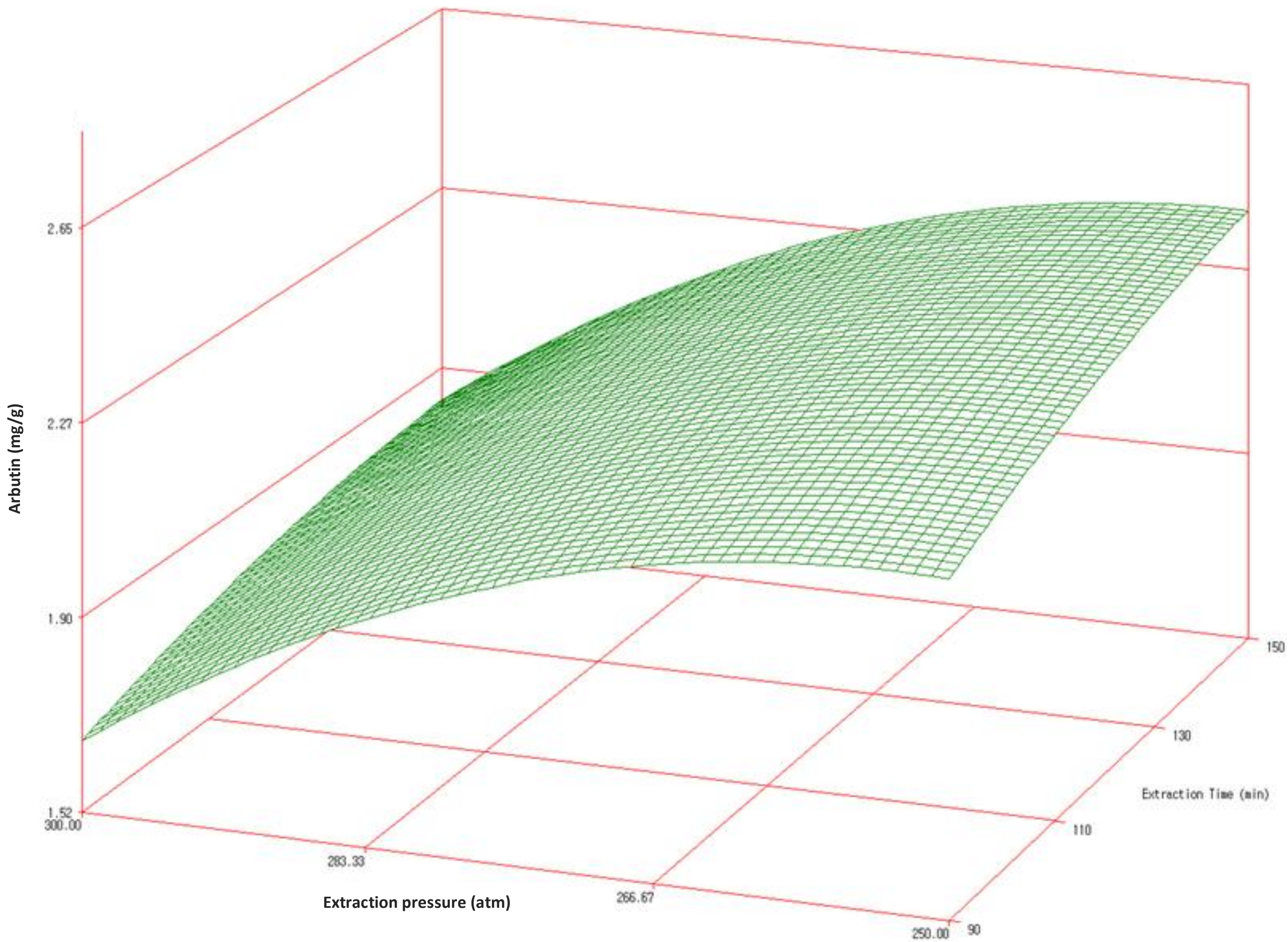

Figure 11. Response surface for the effect of extraction time and extraction pressure on arbutin content of arbutin extracted from Asian pear peel.

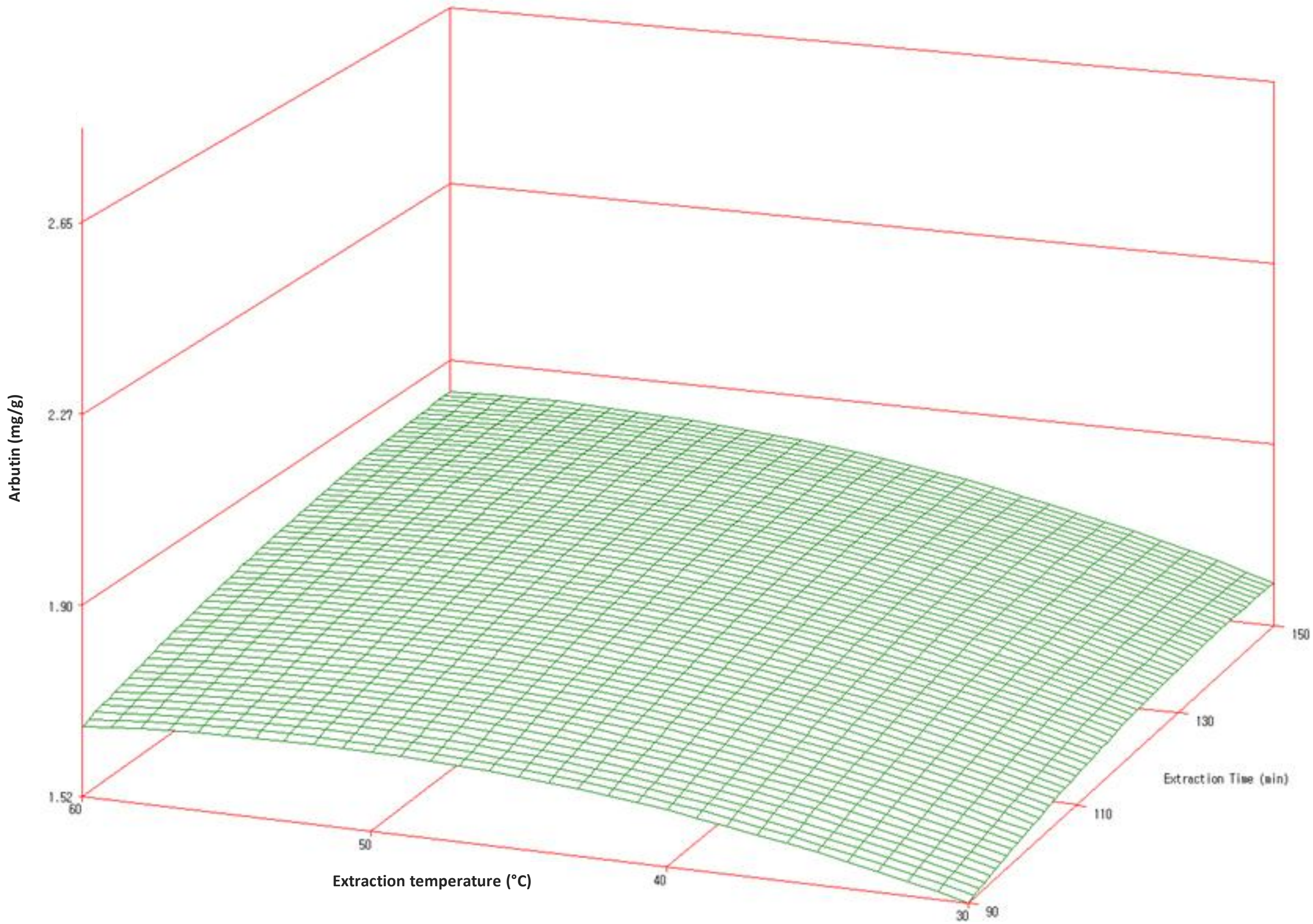

Figure 12. Response surface for the effect of extraction time and extraction temperature on arbutin content of arbutin extracted from Asian pear peel.

was <0.001 and the coefficient of determination (R^2) were 0.84. The variables with a significant effect on arbutin content were the co-solvent concentration (X_1), extraction pressure (X_2), extraction temperature (X_3) and extraction time (X_4) (*P <0.01*).

Conclusion

The effects of the four process variables, that is,co-solvent concentration (22 to 30%), extraction pressure (250 to 300 bars), extraction temperature (30 to 60°C) and extraction time (30 to 60 min), were investigated during the study. Arbutin content by SFE with methanol was the highest with 3.35 mg/g at 26%, 275 bar, 45°C and 45 min.

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