



Research Paper

Optimization of the ultrasonic-assisted extraction process to obtain total phenolic and flavonoid compounds from watermelon (*Citrullus lanatus*) rind

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ABSTRACT

This context presents the study of ultrasonic-assisted extraction (UAE) to obtain phenolic and flavonoid compounds from watermelon rind powder (WRP). The antioxidant activity of the extracts was investigated using DPPH and ABTS⁺ assays. One-factor experiments were conducted to examine the effect of each factor (solid-to-liquid ratio (SLR), acetone concentration (AC), temperature, and time) on the UAE of WRP. Box-Behnken Design (BDD) model was employed to optimize the UAE conditions based on total phenolic contents (TPC), total flavonoid content (TFC), and their antioxidant activities. The optimal conditions were 1:30.50 SLR, 70.71% AC, 29.78 °C, and 10.65 min extraction time. There were no significant differences between predicted and experimental results (less than 6.0%), recommending a feasible and innovative process of deploying UAE to extract phenolics and flavonoids effectively from watermelon rind.

1. Introduction

Research has shown that reactive oxygen species (ROSs) are harmful to human health (Hussain et al., 2016). The excessive generation of ROSs causes lipid oxidation, protein denaturation, and DNA damage by chain-breaking and the polymerization of DNA strains (Rigoulet et al., 2011; Hussain et al., 2016). Currently, various antioxidant products such as anti-aging cosmetics and functional foods are used to neutralize ROS to protect skin and organs (Idha and Gunawan 2013; Balboa et al., 2014). Phenolic compounds are excellent antioxidants because they bond with protein or ions and quench free radicals. Extracts of onions, fruits (apples, grapes, peaches, and strawberries), seeds, roots, and herbs are rich sources of phenolic compounds (Cai et al., 2019; Chua et al., 2019; Kwon et al., 2019; Sridhar and Charles 2019; Wang et al., 2019; Blanco Canalis et al., 2020; Sethi et al., 2020). These extracts are commonly added to antioxidant cosmetics and other personal care products (Ye et al., 2013; Acosta-Estrada et al., 2014; Rähse 2020). In recent years, industrial food waste has been considered as a new source of natural antioxidants, anti-inflammation, and anti-aging agents for

cosmetic products (Faria-Silva et al., 2020). Utilization of this waste can add value to production and reduce environmental treatment costs (Taeymans et al., 2014).

The Cucurbitaceae family includes *Citrullus lanatus* (watermelon), one of the most planned crops in the tropical and temperate zones of the world. The structure of *Citrullus lanatus* consists of three main parts that are the outer layer (peel), rind (mesocarp), and pulp (endocarp). The watermelon rind contains antioxidants and other bioactive compounds such as carotenoids, amino acids, alkaloids, phenolics, and flavonoids (Wehner et al., 2001; Petkowicz et al., 2017).

Traditional techniques (solvent extraction) were commonly employed to obtain bioactive ingredients from plant materials, such as watermelon rind (Chen et al., 2015). However, the drawback of solvent extraction is the excessive use of solvents, which results in low efficiency, low capacity, and high energy consumption (Sharmila et al., 2016). Therefore, several new techniques were conducted to support the extraction process, such as ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE) (Samaram et al., 2015). The disadvantages of the two later

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techniques are the high energy consumption and expensive equipment (Kumar et al., 2021). In contrast, UAE has been considered as a green technology because it can help reduce extraction time and energy consumption (Kumar et al., 2021). UAE has been used to extract bioactive compounds from various materials, such as rice bran, lime peel waste, *Panax notoginseng* flower, fenugreek leaves, gardenia fruits, and mulberry wine waste (Tabaraki and Nateghi 2011; Rodsamran and Sothornvit 2019; Zhang et al., 2020; Huang et al., 2022; Isleroglu and Turker 2022; Wu et al., 2022). However, to our current knowledge, there are no studies on the application of UAE for the recovery of phenolic and flavonoid compounds from watermelon rind.

In this study, Response Surface Methodology (RSM) with a Box-Behnken Design model was employed to find optimal factors of a UAE process, including the effect of solid-to-liquid ratio (SLR), acetone concentration (AC), temperature, and retention time on the extraction efficiency (EE) of phenolic compounds and their antioxidant activities from watermelon rind. Among optimization methodologies, Response Surface Methodology (RSM) allows precise assessment of factorial influences and their interplay. Therefore, it is a practical statistical approach to improving and optimizing UAE processes (Baş and Boyacı, 2007).

2. Materials and methods

2.1. Materials and chemicals

Watermelon rind was obtained from An Nan company, Thanh Hoa, Long An Province, Vietnam, and crushed into fragments. These fragments were dehydrated for 40h at 45 °C and pulverized to obtain watermelon rind powder (WRP). 2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, A1888-2G, purity ≥98%), 1,1-diphenyl-2-picrylhydrazyl (DPPH, 281689-1G, purity ≥97%), Folin-Ciocalteu reagent (F9252-100 ML, concentration 1.9–2.1N), gallic acid monohydrate (398225-100G, purity ≥98%), Whatman Filter Papers No.1 (WHA1001325), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (391913-1G, Trolox, purity 98%), acetone (179124-1L, purity ≥99.5%), sodium carbonate (222321-500G, purity ≥99.5%), potassium acetate (236497-100G, purity ≥99.5%), aluminum chloride hexahydrate (237078-100G, purity 99%), and ethanol (459844-500 ML, purity ≥99.8%) were purchased from Sigma-Aldrich Chemical Co., Ltd, Singapore, Singapore.

2.2. UAE extraction

Phenolic and flavonoid compounds were extracted in an ultrasonic bath Elmasonic (model: S300H, Elma Schmidbauer, Gottlieb-Daimler-Straße, Hohentwiel, Germany), with a maximal volume of 28L (37 kHz, ultrasonic power 300W, total power 1200W). The effect of each factor in the UAE process on the natural compounds and their antioxidant activities were investigated in the following order: SLR, AC, temperature, and time. The sonication of WRP (1 g) was conducted at different SLRs (1:10, 1:20, 1:30, 1:40, and 1:50 g/v), with different ACs (0, 30, 50, 70, and 90%), at different temperatures (20, 30, 40, 50, and 60 °C), for different retention time (5, 10, 30, 50, and 70 min). After extraction, the samples were transferred into a volumetric flask, and distilled water was added until the total volume of 100 ml. The samples were filtered through filter paper. Total phenolic compound (TPC), total flavonoid compound (TFC), DPPH, and ABTS were quantified by the analytical procedures described in section 2.3.

2.3. Total flavonoid content, total phenolic content, and antioxidant activity

TPC was quantified using the Folin-Ciocalteu reagent and TFC using a colorimetric method (UV-vis spectrophotometer, Hach DR/2010, LabWrech, Midland, Ontario, Canada) (Wu et al., 2020) expressed as

milligram of gallic acid equivalent per gram of dried base (mg GAE/g db), and milligram rutin equivalent per gram of dried base (mg Rutin/g db), respectively. DPPH free radical-cleaning activity was performed using an ethanolic-DPPH solution (Müller et al., 2011), and the ABTS method was performed using ABTS⁺ working solution (Müller et al., 2010) expressed as micromol Trolox equivalent per the gram of dried base (µM Trolox/g db)

2.4. Experimental design

The BBD model was employed to optimize the UAE parameters of the WRP. The four independent factors and three levels for the BBD are presented in “Table 1” for the UAE. Four independent factors at three levels (−1, 0, and +1) for 29 experiments were used to measure the response data. The correlation between the response data and the independent factors was determined using a second-order polynomial model utilizing equation (1):

$$Y = B_0 + \sum_{i=1}^k B_i X_i + \sum_{i=1}^k B_{ii} X_i^2 + \sum_{i=1}^k \sum_{j=1}^k B_{ij} X_i X_j \quad (1)$$

where B_0 , B_i , B_{ii} , B_{ij} are the regression coefficients for the intercept, linear, quadratic, and interaction terms, respectively. X_i and X_j represent independent factor values, and k represents the number of independent factors ($k = 4$). The four independent factors and their three levels were as follow: X_1 , SLR: 1:20, 1:30, and 1:40; X_2 , acetone concentration: 50, 70, and 90% v/v; X_3 , temperature: 20, 30, and 40 °C, X_4 , time: 5, 10, and 30 min. Dependent responses (Y) were TPC (mg GAE/g db), TFC (mg Rutin/g db), DPPH (µM Trolox/g db), and ABTS⁺ (µM Trolox/g db).

The prediction error (%) between predicted values and experimental values was calculated by equation (2).

$$\text{Prediction error} = \frac{|\text{the mean of measured value} - \text{predicted values}|}{\text{the mean of measured value}} * 100 \quad (2)$$

2.5. Statistical analysis

All experiments were repeatedly conducted three times and shown as the mean ± SD. Statistical analysis was performed by Statgraphics Centurion 18 (Statgraphics Technologies, Inc, The Plains, Virginia, USA). The BBD model was performed utilizing Design-Expert v.13 software, and the coefficients of the linear, quadratic, and interaction terms were fitted to second-order polynomial regression models. The experimental results were analyzed by analysis of variance (ANOVA) with $\alpha = 0.05$ to test statistically significant differences among the different parameters of the experimental results.

3. Results and discussion

3.1. Effect of solid: liquid ratio

SLR is the main factor in the UAE process for saving solvent and cost, influencing extraction yield (Rao et al., 2021). The effect of SLR on TPC, TFC, DPPH, and ABTS⁺ was investigated and shown in Fig. 1: TPC (Fig. 1A), TFC (Fig. 1B), DPPH (Fig. 1C), and ABTS⁺ (Fig. 1D) from WRP.

Table 1
Experimental design factors and their levels.

Independent factors	Units	The value of independent factors		
		Low (−1)	Middle (0)	High (+1)
X1: SLR	g/v	1:20	1:30	1:40
X2: acetone concentration	%	50	70	90
X3: temperature	°C	20	30	40
X4: time	min	5	10	30

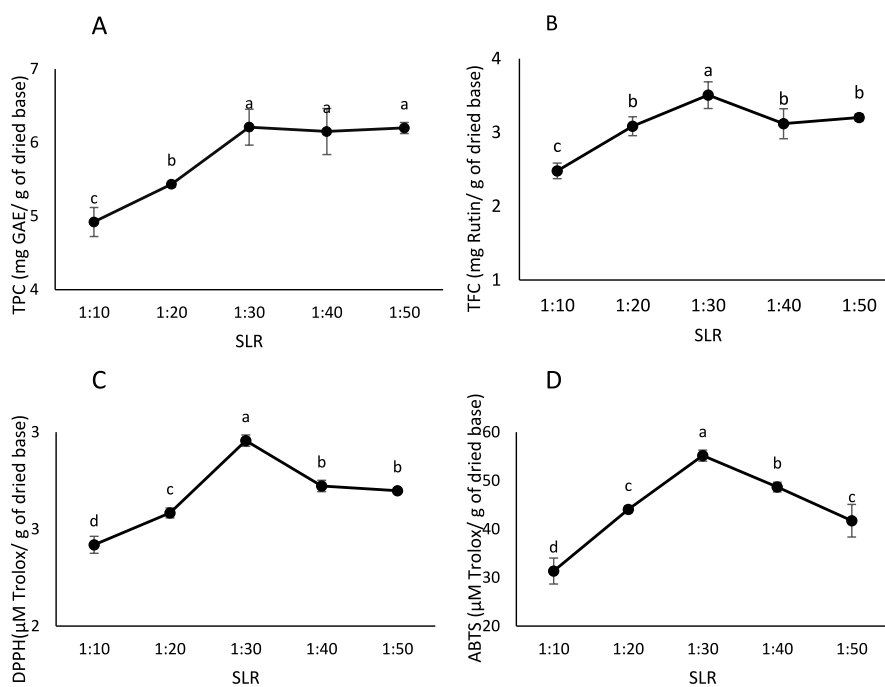


Fig. 1. The effect of SLR on UAE process at regular AC, temperature, time at 70%, 30 °C, 10 min: (A) TPC, (B) TFC, (C) DPPH, (D) ABTS⁺; the characters: a, b, c, d showed significant statistical differences.

As expressed in Fig. 1A and B, TPC and TFC increased by 1.3 and 1.4 times at 1:30 SLR compared to 1:10 SLR. It can be explained that the SLR influences the medium viscosity, thus changing the efficiency of the UAE. When the SLR was lower than 1:10, the viscosity of the medium is high. This phenomenon can hinder the cavitation effect because the negative pressure in the rarefaction region has to surpass the strong

cohesiveness between particles (Rao et al., 2021).

In contrast, when the SLR was higher than 1:10, the decrease in the viscosity of the medium can enhance cavitation, leading to the intensive sponge effect and erosion effect on the WRP surface. However, a further increase in the solvent to solid ratio over 30 times occurred, and the EE of flavonoids decreased to 3.2 ± 0.05 mg Rutin/g db, whereas TPC

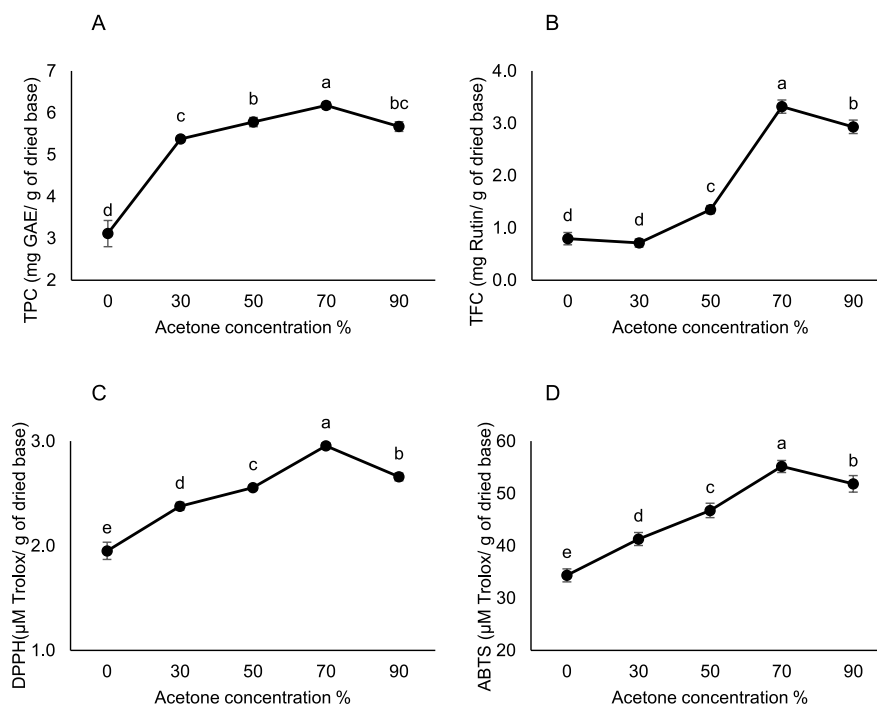


Fig. 2. The effect of acetone concentration AC on UAE process at constant 1:30 SLR, 30 °C, and 10 min retention time: (A) TPC, (B) TFC, (C) DPPH, (D) ABTS⁺; the characters: a, b, c, d showed significant statistical differences.

remained unchanged. It can be attributed to the more substantial cavitation that causes the disintegration of flavonoids (Kumar et al., 2021). Having a similar trend with TFC, DPPH and ABTS⁺ represented the antioxidant activities of phenolic and flavonoid compounds in WRP, which peaked at $2.96 \pm 0.03 \mu\text{M}$ Trolox/g db and $55.18 \pm 1.16 \mu\text{M}$ Trolox/g db, respectively. It can be justified that TPC and TFC positively correlate with higher antioxidant activity, such as DPPH and ABTS⁺ (Mahindrakar and Rathod 2020). Therefore, 1:30 SLR was chosen as the suitable ratio for obtaining the optimal TPC and TFC at $6.21 \pm 0.24 \text{ mg GAE/g db}$ and $3.51 \pm 0.18 \text{ mg Rutin/g db}$, respectively.

3.2. Effect of acetone concentration

The variation of AC changes solution polarity, which may play a vital role in EE (Muñiz-Márquez et al., 2013). Fig. 2 shows the effect of AC in the range of 0–90% on the EE of phenolics and flavonoids. The highest TPC and TFC were observed at 70% AC, which was 2.0 and 4.9 times higher than 0%, respectively. Increasing the AC to 70% can reduce the polarity of the solution and enhance the solubility of phenolics and flavonoids in WRP. In addition, the appropriate water content in the solution probably triggers the swelling of WRP and increases the contact area between solvent and solute. Those can be the explanation for the improved EE of phenolics and flavonoids when changing the AC (Muñiz-Márquez et al., 2013). However, when AC increased to 90%, TPC and TFC decreased. High AC can cause protein denaturation and pectin dehydration that impede the diffusion of phenolics and flavonoids in the WRP matrix toward the medium (Muñiz-Márquez et al., 2013). The same trend was observed for DPPH and ABTS⁺, which peaked at $2.96 \pm 0.03 \mu\text{M}$ Trolox/g db and $32.88 \pm 0.32 \mu\text{M}$ Trolox/g db, respectively. As a result, AC of 70% was suitable for obtaining the optimal TPC at $6.17 \pm 0.09 \text{ mg GAE/g db}$ and TFC at $3.32 \pm 0.13 \text{ mg Rutin/g db}$.

3.3. Effect of temperature

Traditional solvent extraction tends to be conducted at high

temperatures to facilitate the mass transfer rate and cavitation effect to improve phenolic and flavonoid solubility (Rao et al., 2021). Fig. 3 shows the effect of temperature varied from 20 to 60 °C on TPC (Fig. 3A), TFC (Fig. 3B), DPPH (Fig. 3C), and ABTS⁺ (Fig. 3D). The highest TPC and TFC were found at 30 °C at $6.21 \pm 0.24 \text{ mg GAE/g db}$ and $3.51 \pm 0.18 \text{ mg Rutin/g db}$, respectively. At higher temperatures, the solubility and diffusivity of phenolics and flavonoids are improved, enhancing the mass transfer, thus increasing the EE of these compounds (Mahindrakar and Rathod 2020). However, the TPC decreased by 1.15 times, whereas the TFC remained unchanged with the growth of extraction temperature from 30 to 60 °C. It is possible that heat can reduce the differences in vapor pressure between the inside and outside of the cavitation bubbles, thereby decreasing the intensity of collapsing bubbles (Mahindrakar and Rathod 2020). Moreover, an increase in temperature can cause a drop in surface tension, declining the shear force of exploding bubbles on the WRP surface. The heat probably causes phenolic deterioration, thus reducing the DPPH and ATBS values of WRP extract (Kumar et al., 2021). Hence, 30 °C was a suitable temperature for achieving the optimal TPC, TFC, DPPH, and ABTS⁺ at $6.21 \pm 0.24 \text{ mg GAE/g db}$, $3.51 \pm 0.18 \text{ mg Rutin/g db}$, $2.96 \pm 0.03 \mu\text{M}$ Trolox/g db, and $55.18 \pm 1.16 \mu\text{M}$ Trolox/g db, respectively.

3.4. Effect of time

Time plays an essential role in minimizing the running cost of the extraction process (Rao et al., 2021). Fig. 4 shows the effect of time from 5 to 70 min on TPC (Fig. 4A), TFC (Fig. 4B), DPPH (Fig. 4C), and ABTS⁺ (Fig. 4D). Initially, TPC and TFC were escalated within the first 10 min and started to decrease after. The initial higher EE can be owing to the higher slope of the gradient solvent, which decreases by time. Additionally, short extraction time is reached due to cavitation, thermal and physical effects generated at the WRP surface (Mahindrakar and Rathod 2020).

On the other hand, an excessive increase in time can decrease TPC, TFC, DPPH, and ABTS⁺. Long time exposure to ultrasonic waves could trigger the degradation of phenolics and flavonoids, leading to the

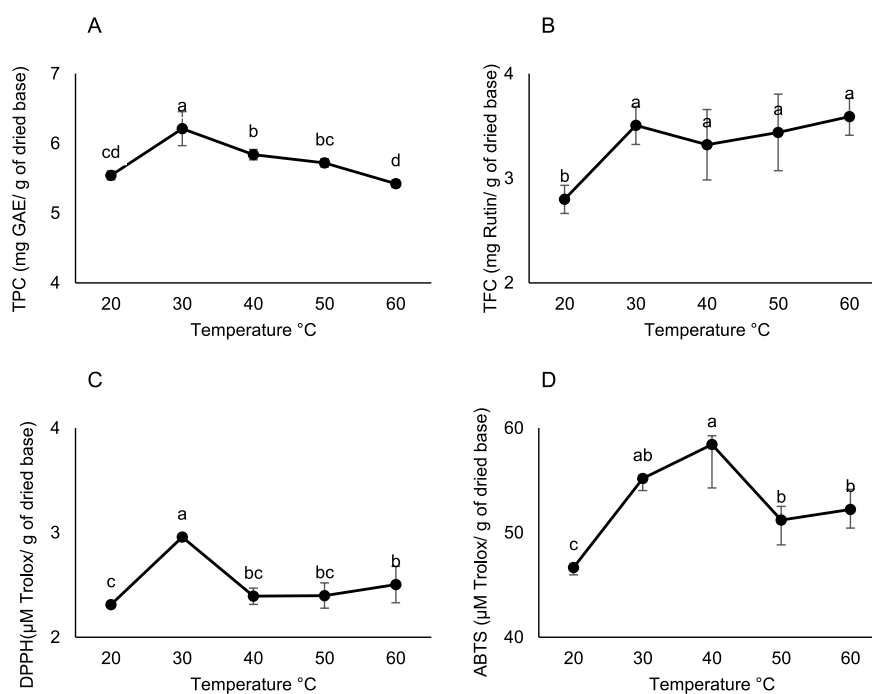


Fig. 3. The effect of temperature on UAE process at constant SLR, acetone concentration AC, time at 1:30, 70%, 10 min: (A) TPC, (B) TFC, (C) DPPH, (D) ABTS⁺; the character: a, b, c, d showed significant statistical differences.

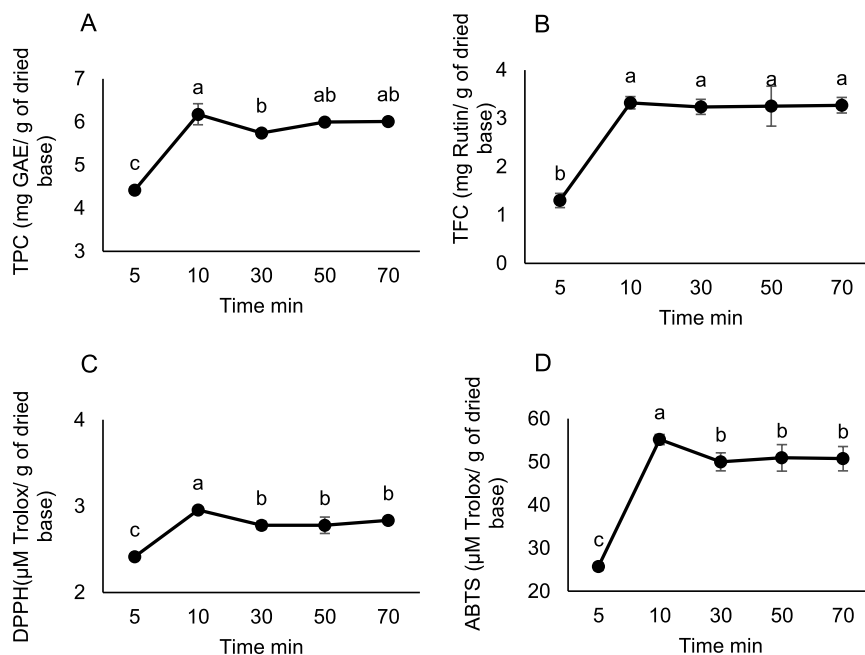


Fig. 4. The effect of time on UAE process at constant SLR, acetone concentration, temperature at 1:30, 70%, 30 °C: (A) TPC, (B) TFC, (C) DPPH, (D) ABTS⁺; the characters: a, b, c, d showed significant statistical differences.

decrease in DPPH value at $2.78 \pm 0.05 \mu\text{M Trolox/g db}$ and ABTS⁺ value at $30.92 \pm 0.51 \mu\text{M Trolox/g db}$ at 30 min. Therefore, 10 min was chosen as the appropriate retention time for extracting phenolics and flavonoids and retaining the antioxidant activity of extracts.

3.5. Optimization of UAE process for WRP

The conditional range of independent factors was chosen from the experimental results of UAE conditions in sections 3.1, 3.2, 3.3, and 3.4; the range consisted of three values: proper, upper-proper, and lower-proper conditions (coded 0, 1, and -1, respectively). The results

Table 2
BBD design and the results of experiments for quantified responses with WRP.

Run	Factor				TPC		TFC		DPPH		ABTS ⁺	
	X1	X2	X3	X4	Predicted Values	Experimental Values	Predicted Values	Experimental Values	Predicted Values	Experimental Values	Predicted Values	Experimental Values
1	0	0	0	0	6.16	6.17 ± 0.09	3.29	3.32 ± 0.13	2.96	2.95 ± 0.03	55.18	55.18 ± 0.94
2	0	0	1	-1	5.25	5.21 ± 0.24	2.55	2.41 ± 0.15	2.58	2.54 ± 0.09	48.38	49.90 ± 1.59
3	-1	0	-1	0	5.16	5.04 ± 0.13	1.48	1.49 ± 0.10	2.60	2.62 ± 0.10	43.89	42.49 ± 0.35
4	0	0	0	0	6.16	6.17 ± 0.09	3.29	3.32 ± 0.13	2.96	2.95 ± 0.03	55.18	55.18 ± 0.94
5	-1	0	1	0	5.39	5.32 ± 0.14	1.86	1.89 ± 0.31	2.57	2.49 ± 0.09	45.97	44.62 ± 1.51
6	0	-1	0	-1	4.82	4.68 ± 0.05	2.11	2.01 ± 0.15	2.48	2.53 ± 0.02	42.31	40.27 ± 0.47
7	0	-1	-1	0	4.60	4.62 ± 0.23	1.12	1.22 ± 0.05	2.30	2.21 ± 0.13	35.34	35.69 ± 3.52
8	0	1	0	-1	4.93	4.91 ± 0.11	2.99	2.96 ± 0.20	2.67	2.72 ± 0.07	48.94	48.69 ± 1.04
9	-1	0	0	1	5.33	5.38 ± 0.22	2.17	2.27 ± 0.13	2.87	2.94 ± 0.02	53.52	53.73 ± 0.92
10	1	0	0	1	6.09	5.93 ± 0.54	2.82	2.81 ± 0.31	2.93	2.91 ± 0.07	50.72	49.08 ± 1.77
11	1	0	0	-1	5.34	5.27 ± 0.04	2.68	2.79 ± 0.29	2.53	2.44 ± 0.01	49.80	48.42 ± 0.99
12	0	0	0	0	6.16	6.17 ± 0.09	3.29	3.32 ± 0.13	2.96	2.96 ± 0.03	55.18	55.18 ± 0.94
13	-1	-1	0	0	4.76	4.72 ± 0.05	1.39	1.23 ± 0.09	2.60	2.59 ± 0.01	48.60	49.55 ± 1.44
14	0	1	1	0	5.27	5.22 ± 0.07	2.02	2.14 ± 0.27	2.46	2.53 ± 0.05	42.40	40.88 ± 0.57
15	-1	1	0	0	5.34	5.38 ± 0.08	1.98	1.8 ± 0.080	2.72	2.69 ± 0.05	41.22	42.34 ± 1.25
16	1	0	1	0	6.07	6.18 ± 0.20	1.92	1.87 ± 0.13	2.55	2.56 ± 0.07	42.42	43.06 ± 0.92
17	-1	0	0	-1	5.23	5.37 ± 0.11	2.36	2.58 ± 0.08	2.84	2.84 ± 0.02	53.23	53.69 ± 0.91
18	0	1	0	1	5.44	5.57 ± 0.09	3.02	3.08 ± 0.05	2.79	2.77 ± 0.05	48.34	49.62 ± 1.25
19	0	0	-1	-1	4.95	5.09 ± 0.15	2.51	2.47 ± 0.24	2.42	2.42 ± 0.04	42.91	44.56 ± 3.94
20	0	0	1	1	5.83	5.72 ± 0.16	2.44	2.34 ± 0.06	2.71	2.68 ± 0.03	45.16	45.42 ± 0.57
21	0	0	0	0	6.16	6.12 ± 0.09	3.29	3.19 ± 0.13	2.96	2.96 ± 0.03	55.18	55.18 ± 0.94
22	0	-1	0	1	5.15	5.17 ± 0.16	2.02	2.01 ± 0.10	2.80	2.77 ± 0.05	44.12	43.60 ± 0.69
23	0	0	0	0	6.16	6.17 ± 0.09	3.29	3.32 ± 0.13	2.96	2.96 ± 0.03	55.18	55.18 ± 0.94
24	0	1	-1	0	5.09	4.91 ± 0.14	2.80	2.84 ± 0.08	2.46	2.4 ± 0.050	37.98	36.38 ± 0.69
25	1	1	0	0	5.40	5.47 ± 0.12	2.81	2.82 ± 0.31	2.57	2.54 ± 0.02	50.90	51.84 ± 2.18
26	0	0	-1	1	5.21	5.28 ± 0.15	2.57	2.57 ± 0.08	2.73	2.73 ± 0.10	47.34	47.71 ± 1.76
27	0	-1	1	0	5.36	5.51 ± 0.07	1.81	1.98 ± 0.18	2.44	2.49 ± 0.07	34.20	34.62 ± 0.69
28	1	-1	0	0	5.57	5.56 ± 0.16	1.52	1.55 ± 0.13	2.51	2.51 ± 0.04	32.68	33.45 ± 1.25
29	1	0	-1	0	5.35	5.41 ± 0.27	2.38	2.31 ± 0.08	2.38	2.48 ± 0.06	41.20	41.79 ± 3.02

obtained from the UAE of WRP are shown in Table 2, and the regression coefficients are shown in Table 3. Significant coefficients (Table 3) were selected to formulate regression models (Equation (3)–(6)) to forecast the response values. The forecasting models were significantly suitable for F-values of 26.07, 44.03, 19.26, and 40.95 for TPC, TFC, DPPH, and ABTS⁺, respectively. The large values of R² (0.9506–0.9779) speculated a significant statistical model. SLR and AC positively correlated with the dependent responses, namely TPC, TFC, DPPH, and ABTS⁺. 3D response surface graphics (Figs. 5 and 6) illustrate the correlation between dependent responses and independent factors; such graphics indicate the responses of the two factors and retain other factors unchanged at their middle level.

$$Y_{TPC} = 6.16 + 0.22X_1 + 0.1X_2 + 0.23X_3 + 0.21X_4 - 0.19X_1X_2 + 0.16X_1X_4 - 0.15X_2X_3 - 0.24X_1^2 - 0.65X_2^2 - 0.43X_3^2 - 0.42X_4^2 \quad (3)$$

$$Y_{TFC} = 3.29 + 0.24X_1 + 0.47X_2 + 0.17X_1X_2 - 0.21X_1X_3 - 0.37X_2X_3 - 0.7X_1^2 - 0.67X_2^2 - 0.68X_3^2 \quad (4)$$

$$Y_{DPPH} = 2.96 - 0.06X_1 + 0.05X_2 + 0.11X_4 + 0.09X_1X_4 - 0.13X_1^2 - 0.23X_2^2 - 0.31X_3^2 \quad (5)$$

$$Y_{ABTS} = 55.18 - 1.56X_1 + 2.71X_2 + 6.4X_1X_2 - 1.91X_3X_4 - 2.97X_1^2 - 8.86X_2^2 - 8.84X_3^2 \quad (6)$$

The second-order polynomial equations (3) and (4) show the correlation of TPC and TFC, respectively with independent factors. The linear influence of acetone concentration AC and temperature significantly impacted TPC, which relied on X₂², X₃², X₄², X₃, and X₁₂ (Table 3) based on regression coefficients. It was discovered that the interaction between SLR and AC was negative, whereas the interactive effect of SLR and time was positive. 3D response surface plots (Fig. 5A1–A6) were used to describe the interactive influences of four independent factors on the EE of phenolics from WRP. SLR and AC expressed the interactive effect on the EE of phenolics. TPC grew to 6.18 mg GAE/g db with increasing SLR and AC, followed by a slight decrease (Fig. 5A1). The results had an agreement with the findings that Reza Tabaraki and Ashraf Nateghi

Table 3
Regression coefficients for the quadratic models of all independent factors and the information on UAE optimization.

	Coefficient	TPC	TFC	DPPH	ABTS ⁺
Intercept	B ₀	6.16**	3.29**	2.96**	55.18**
Linear	B ₁	0.22*	0.24**	-0.06**	-1.56**
	B ₂	0.10**	0.47**	0.05*	2.71**
	B ₃	0.23**	-0.02 ^{ns}	0.04 ^{ns}	0.82 ^{ns}
	B ₄	0.21*	-0.01 ^{ns}	0.11**	0.30 ^{ns}
Interaction	B ₁₂	-0.19*	0.17*	-0.02 ^{ns}	6.40**
	B ₁₃	0.12 ^{ns}	-0.21**	0.05 ^{ns}	-0.21 ^{ns}
	B ₁₄	0.16*	0.08 ^{ns}	0.09*	0.16 ^{ns}
	B ₂₃	-0.15*	-0.37**	-0.04 ^{ns}	1.39 ^{ns}
	B ₂₄	0.04 ^{ns}	0.03 ^{ns}	-0.05 ^{ns}	-0.60 ^{ns}
	B ₃₄	0.08 ^{ns}	-0.04 ^{ns}	-0.04 ^{ns}	-1.91*
	B ₁₂₃	-0.24**	-0.70**	-0.13**	-2.97**
Quadratic	B ₂₂	-0.65**	-0.67**	-0.23**	-8.86**
	B ₃₃	-0.43**	-0.68**	-0.31**	-8.84**
	B ₄₄	-0.42**	-0.09 ^{ns}	-0.04 ^{ns}	-0.39 ^{ns}
	Degree of freedom	14	14	14	14
F-values		26.07	44.30	19.26	40.95
p-values		<0.0001	<0.0001	<0.0001	<0.0001
R ²		0.9631	0.9779	0.9506	0.9762
R ² _{adjusted}		0.9261	0.9559	0.9013	0.9523

Notes: ns: not significant (p > 0.05); *: significant (p < 0.05); **: highly significant (p < 0.01).

reported about the EE of phenolics from rice bran using UAE being 6.35 mgGAE/g db (Tabaraki and Nateghi 2011).

The linear influence of the SLR and AC show the significant impacts on the TFC that primarily depended on X₁², followed by X₃², X₂² and X₂ (Table 3). Regression coefficients also illustrate that AC had a higher impact on TFC than SLR due to B₁ < B₂ (0.24 < 0.47). 3D response surface graphics were built to imagine the interactive influence of the four independent factors on the EE of flavonoids from WRP (Fig. 4B1–B6). The SLR and AC had positive interactions with TFC. TFC rose with increasing AC, and SLR peaked at the maximal value of 3.34 mg Rutin/g db, followed by a slight reduction.

Moreover, the AC had a more substantial effect on the extraction of flavonoids than phenolics, which could be attributed to the lower polarity of flavonoids compared to phenolics. The polar comparison was simplified using two standard substances: quercetin and gallic acid, owing to a wide variety of phenolic and flavonoid compounds and having similar chemical structures. The partition coefficients (XLOGP3, a lipophilicity index) are used to forecast the hydrophobicity/hydrophilicity of molecules (Carrasco-Pozo et al., 2012). According to the PubChem database, gallic acid and quercetin have partition coefficients of 0.7 and 1.5, respectively; thus, quercetin has more lipophilicity than gallic acid. Furthermore, increasing the AC decreases solution polarity, which can be approximate to flavonoid polarity because acetone has a lower relative polarity index (0.355) than water (1) (Reichardt and Welton 2010). Therefore, the extractability of flavonoids in a high AC can be higher than that of phenolics.

Table 3 shows that the models for DPPH and ABTS⁺ were primarily significant (p < 0.01). The DPPH was significantly impacted by X₄, X₃², X₂², and X₁X₄. The mutual effect between SLR and time significantly affected DPPH (p < 0.05). 3D response surface graphics were constructed to visualize the influence of the four independent factors on the DPPH of WRP (Fig. 6C1–C6). It can be shown that SLR and time positively influenced DPPH. The DPPH increased with the increasing SLR and time achieved the maximal values at 2.96 μM Trolox/g db, followed by a moderate reduction. Although the antioxidant activity of WRP was lower than that of rice bran, WRP could be more economical and available, contributing to a circular economy (Tabaraki and Nateghi 2011).

ABTS⁺ was greatly influenced by X₂², X₃², X₁X₂, X₁², and X₂². SLR and AC profoundly impacted ABTS⁺, whereas SLR and time showed no significant effect. 3D response surface plots were drawn to visualize the influence of four independent factors on the ABTS⁺ of WRP (Fig. 6D1–D6). X₂, X₃, and X₄ positively affected ABTS⁺, whereas X₁², X₂², and X₃² had a negative relationship. A similar trend was found in the UAE in lime peel waste (Rodsamran and Sothornvit 2019) and *Moringa oleifera* L. leaves (Wu et al., 2020). From all response models, the optimal conditions of UAE for phenolics and flavonoids recovery from WRP were using 1:30.50 of SLR, 70.71% AC 29.78 °C, and an extraction time: of 10.65 min.

3.6. Model verification

Table 4 shows the experimental values of the dependent responses under the optimal conditions in the UAE process. The reliability of the BBD models was verified by performing experimental verification under the optimal conditions of the UAE process, which were selected through 3D surface plots and regression analysis of independent factors. The optimum UAE conditions were chosen at 1:30.50 of SLR, 70.71% AC, 29.78 °C, and an extraction time: of 10.65 min. The predicted values of TPC, TFC, DPPH and ABTS⁺ were 6.18 mg GAE/g db, 3.34 mg Rutin/g db, 2.96 μM Trolox/g db, and 55.25 μM Trolox/g db respectively. It can be noted that predicted values were well fitted with experimental values with low prediction errors (<5.70%).

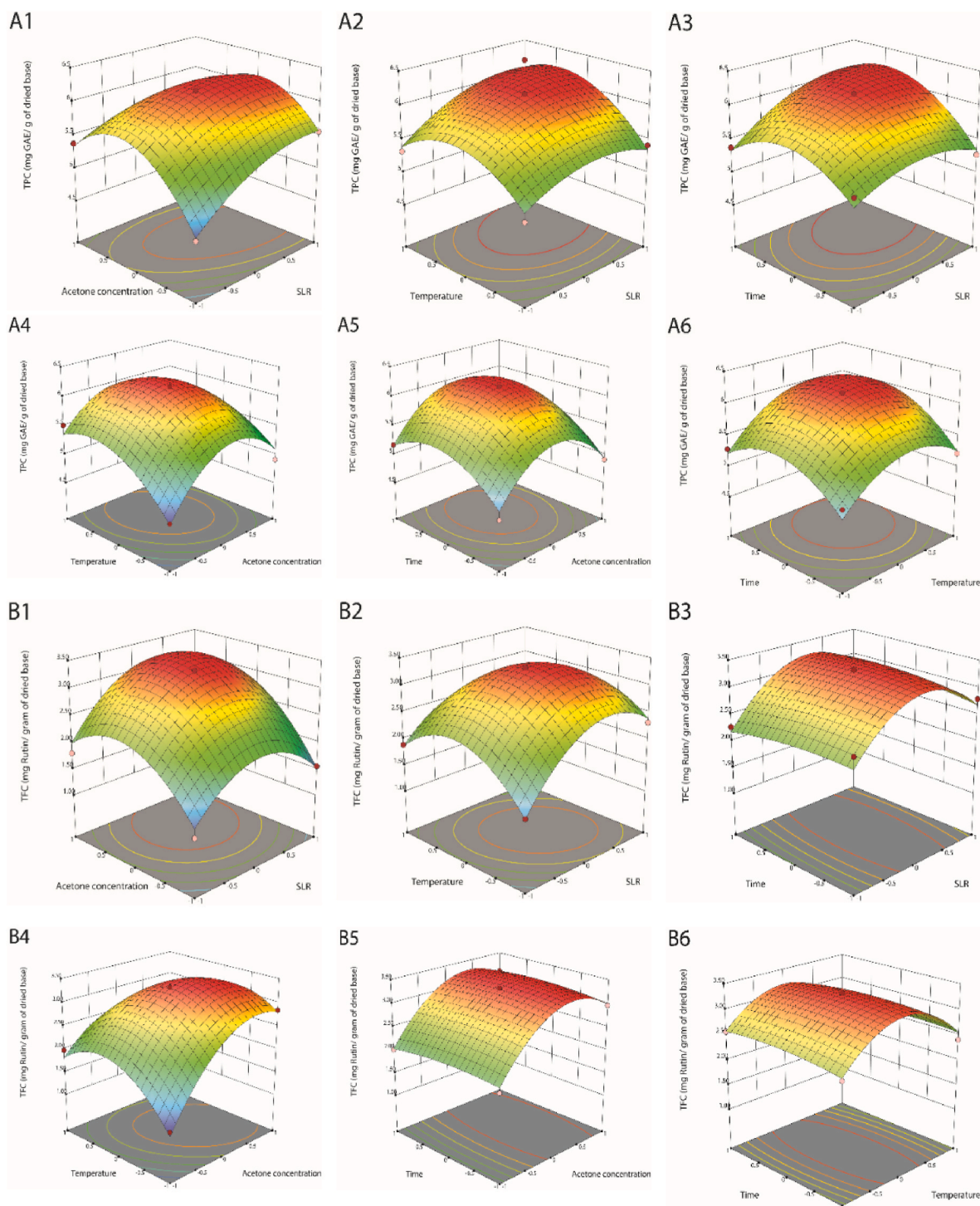


Fig. 5. 3D response surface plots demonstrate the interactive effects of independent factors: SLR, AC, temperature, and time on the recovery of TPC (A1–A6) and TFC (B1–B6).

4. Conclusion

The temperature was the most crucial factor affecting TPC, while AC was that of TFC with high antioxidant activity for UAE of WRP. The optimal UAE conditions were at 1:30.50 of SLR, 70.71% AC, 29.78 °C, and an extraction time: of 10.65 min. The highest values of the determination coefficients ($R^2 > 0.95$) verified the suitability of the predicted models, and the distinction between the predicted and experimental values was not significant. This study demonstrated that ultrasonic-assisted extraction is a green and efficient method to recover large

amounts of phenolics and flavonoids from watermelon rind powder that can be considered a valuable source of antioxidants.

CRediT authorship contribution statement

Tan Phat Vo: Conceptualization, Methodology, Investigation, Software, Formal analysis, Writing – original draft. **Le Ngoc Huong Nguyen:** Investigation, Visualization. **Nguyen Phuc Thien Le:** Investigation. **Thanh Phong Mai:** Visualization. **Dinh Quan Nguyen:** Visualization, Supervision, Writing – review & editing.

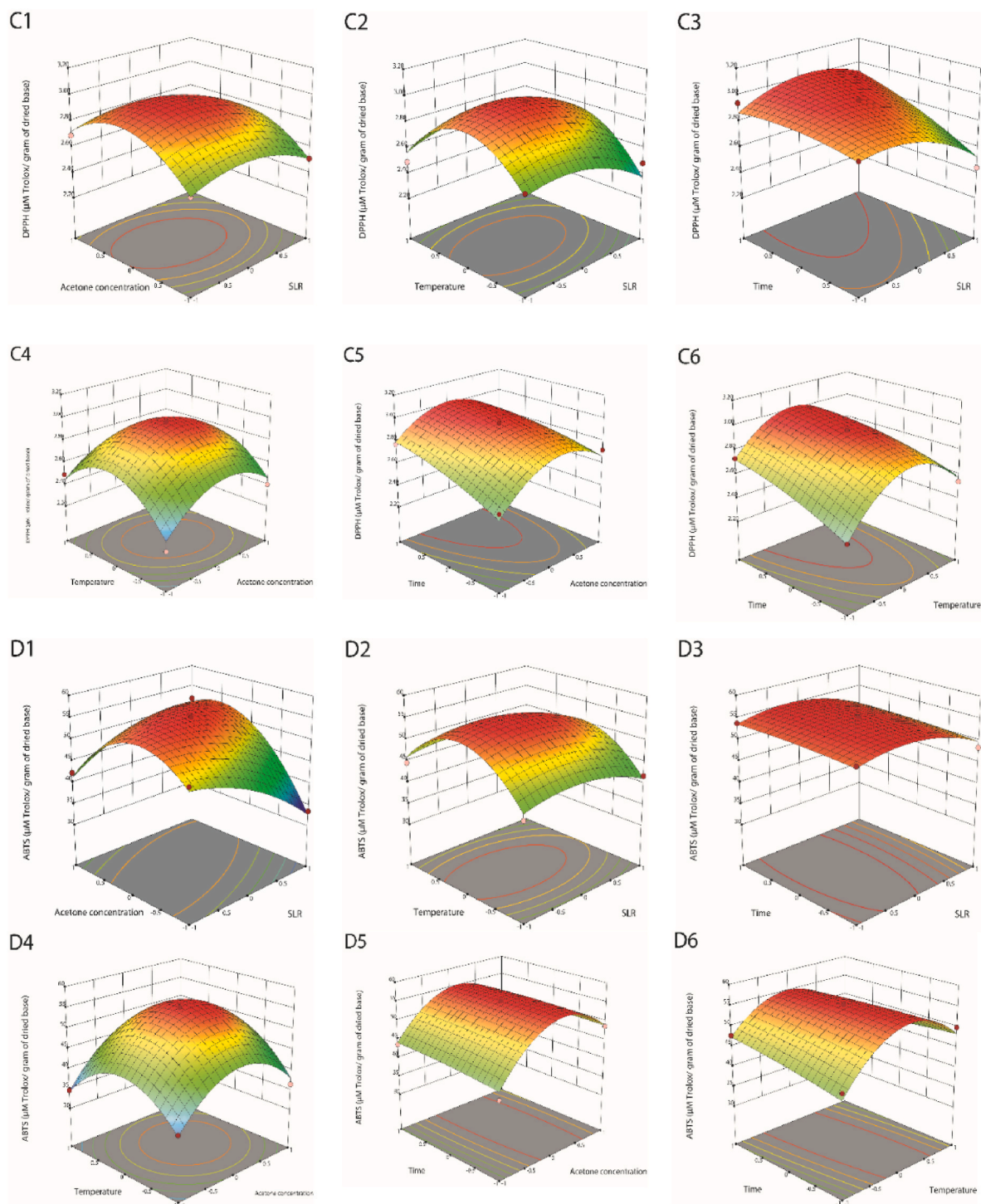


Fig. 6. 3D response surface plots demonstrate the interactive effects of independent factors: SLR, AC, temperature, and time on DPPH (C1–C6) and ABTS⁺ (D1–D6).

Table 4
Actual and predicted values of TPC, TFC, DPPH, and ABTS⁺ at the optimal conditions in the UAE process.

The independent factors of the UAE				Dependent responses				
SLR	Acetone concentration %	Temperature °C	Time min		Predicted values	Experimental values	Prediction error %	R ² _{predicted}
1:30:50	70.71	29.78	10.65	TPC (mg GAE/g db)	6.18	6.31 ± 0.14	2.06	0.7885
				TFC (mg Rutin/g db)	3.34	3.16 ± 0.08	5.70	0.8778
				DPPH (μM Trolox/g db)	2.96	3.07 ± 0.03	3.58	0.7161
				ABTS ⁺ (μM Trolox/g db)	55.25	56.20 ± 1.39	1.69	0.8627

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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