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Improvement and Optimisation of Green Ultrasound-Assisted Extraction for Sinapic Acid Content and Lipase Inhibition From Caucasian Whortleberry (*Vaccinium arctostaphylos* L.) by Box–Behnken Design

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ABSTRACT

Caucasian whortleberry (*Vaccinium arctostaphylos* L.) is a beneficial natural source for obesity due to its rich content of phenolic compounds. It has been shown that phenolic compounds have antiobesity properties through a variety of molecular mechanisms. The goal of the current study was to identify the optimal conditions of green ultrasonic extraction (UAE) for Caucasian whortleberry via phenolic compounds and lipase inhibition. The experimental design was carried out using the response surface methodology (RSM) based on the Box–Behnken design (BBD) to detect optimum conditions of ultrasonic extraction. Three levels of three independent variables were incorporated into the BBD: ethanol concentration, temperature and time. RP-HPLC analysis was utilised to quantify the phenolic content, and the spectroscopic method was used to evaluate the lipase inhibitions. Quadratic response surface models were suggested according to the results of the BBD model adequacy test ($p < 0.0001$) performed by multiple regression analysis. The data showed that the sinapic acid concentration and lipase inhibition are significantly impacted by the extraction conditions. The optimal conditions for sinapic acid content and lipase inhibition were detected as 100% ethanol concentration, 60°C and 60 min. The highest sinapic acid content (13.66 mg/g dry extract) and lipase inhibition level ($IC_{50} = 66.22\,\mu g/mL$) with desirability of 0.8583 resulted under optimal conditions. In comparison with the conventional extraction method, optimal conditions resulted in a notable rise of 57.01% for sinapic acid content and a substantial increase of 53.12% for lipase inhibitory effect. These optimal conditions mediated more sinapic acid content and lipase inhibitor activity can be suggested for the development of food supplements or herbal medicine with Caucasian whortleberry.

1 | Introduction

Vaccinium genus is represented by approximately 450 species distributed through Asia, South East and Central Africa, Europe and North and Central America [1]. Anthocyanins and phenolic compounds have been valued to be the *Vaccinium* genus' major secondary metabolites by various phytochemical investigations [2]. *Vaccinium* genus has been known for its bioactive qualities, which include the potential to treat or prevent ageing-related disorders, cancer, diabetes, urinary tract infections and obesity [2–4].

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The extraction process and method optimisation are crucial for achieving a high concentration of bioactive compounds from natural sources [5]. Design of experiment (DOE) is a widely used method of determining the relationship between factors that affect the results of a process. DOE has advantages such as reducing the number of experiments, defining the interaction between factors and optimising the experimental method [6]. Response surface methodology (RSM) is a significant analytical technology that utilises the DOE, to search for an appropriate relationship between variables and to determine the best operating conditions for the process. One of the main experimental designs used in RSM is the Box–Behnken design (BBD) [7]. BBD is frequently utilised to optimise the recovery of bioactive compounds in pharmaceutical applications. It offers numerous benefits, including the use of blocks, the absence of model fit detection, the capacity to create sequential designs and the estimate of the quadratic model's parameters. The major benefit of this design above others is that it does not require conducting experiments in situations that could be expensive or difficult to test, such as employing the lowest or highest amounts of all the components concurrently [1].

The application of BBD in herbal extracts has been the subject of much scientific research. BBD was used to obtain the fraction of Rhamnus alaternus rich in bioactive metabolites such as polyphenols and flavonoids [8]. The extract of Leontodon hispidulus prepared using the BBD was found to increase the content of phenolic compounds as well as anti-inflammatory and anticancer activity [1]. The content of phenolic compounds in olive leaf extract, whose optimum conditions were determined using the BBD, also increased compared with traditional extraction methods [9]. Extraction of *Malva parviflora* L. leaves under optimal conditions determined using BBD mediated an increase in phenolic compounds content and antioxidant activity [10].

Obesity is one of today's most challenging chronic illnesses, and both its prevalence and comorbidities are increasing globally [11]. Pancreatic lipase inhibition, which reduces the absorption of lipids, is one of the effective therapeutic strategies for the treatment of obesity. Pancreatic lipase inhibitors of natural origin have grown in popularity since they are less likely to produce side effects than drugs of synthetic origin. It has been discovered that phenolic compounds in natural resources contribute to the antiobesity effects [11, 12].

It is widely acknowledged that green ultrasonic extraction (UAE) is a very effective and ecologically friendly extraction technique. UAE can reduce its environmental impact by minimising or eliminating the requirement for organic solvents. The method has remarkable benefits in obtaining bioactive compounds from plants. The method's cavitation property enables to extraction of selected bioactive components effectively. This novel method improves the quality and yield of the extract while decreasing the extraction time. Thus, the UAE has the potential to create novel opportunities for research in the fields of food and the pharmaceutical industry [13]. Ultrasound exerts a considerable impact on the phytochemical composition of plants, particularly in relation to phenolic compounds. For instance, employing ultrasound for the extraction of gingerols from ginger powder at a temperature of 50°C improved the yield of gingerol. The use of UAE resulted in a 20% increase in α-tocopherol levels and a 44% enhancement in total phenolic content when compared to conventional methods. As a result, phenolic compounds are widely extracted from diverse plant sources through UAE, owing to its advantages such as decreased solvent consumption, lower environmental impact, reduced extraction time and improved penetration efficiency [14, 15].

To the best of our knowledge, there is no study targeted optimisation of green extraction for Caucasian whortleberry in terms of phenolic compounds and lipase inhibition. Thus, the present study was designed to optimise the green extraction process in terms of phenolic compounds and lipase inhibition for Caucasian whortleberry by using RSM utilising BBD.

2 | Materials and Methods

2.1 | Plant Material

The plant material for the study was collected from Ormancık Village, Akkuş, ORDU region, at an altitude of 1190 m. The voucher specimen (AEF 26969) was deposited at the Herbarium of the Faculty of Pharmacy, Ankara University.

2.2 | Optimisation of Extraction Conditions

2.2.1 | Design of Experiment

The optimisation study was carried out using a factorial-based Box–Behnken design (BBD, 3-factor, 3-level) (Design Expert V.8) [1]. Two responses (lipase inhibition, sinapic acid content) as dependent variables and three factors (solvent ratio, temperature and time) as independent variables were examined. The factors examined and their respective levels were selected in accordance with previous research [16–19]. The coded and related values are listed in Table 1. The design had a total of 17 runs, five of which were centre points. The extraction conditions of 17 runs are displayed in Table 2.

2.2.2 | Preparation of Extracts

According to the optimisation research design shown in Table 2, the powder of the fruits of wild blueberries, weighing about 1 g for each run, was extracted by ultrasonic extraction using various ratios of ethanol/water (100%, 50%, 0%), temperature (40°C, 60°C, 80°C) and time (20, 40, 60 min).

Also, the maceration technique was employed with the same ethanol/water ratio (100%, 50%, 0%) at room temperature for a day to compare with the green extraction technique with the conventional technique. All extracts prepared with the green and conventional technique were evaporated under reduced pressure at 40°C to obtain crude extracts.

2.3 | HPLC Conditions

Our previous method was used to perform HPLC analysis [20]. Zorbax C18 column (150 × 4.6 mm, 5 μm) was used with Shimadzu Corporation, LC 20AT, Kyoto, Japan for HPLC analysis. The flow rate was 1.5 mL/min, and the injection volume was

TABLE 1 | Independent and dependent variables of BBD Analysis.

Independent variables					
Factors	**Name**	**Units**	**Coded low**	**Coded mean**	**Coded high**
A	Solvent ratio*	%	−1 ↔ 0.00	0 ↔ 50.00	+1 ↔ 100.00
B	Temperature	°C	−1 ↔ 40.00	0 ↔ 60.00	+1 ↔ 80.00
C	Time	min	−1 ↔ 20.00	0 ↔ 40.00	+1 ↔ 60.00
Dependent variables					
Responses	**Name**	**Units**			
R1	Lipase inhibition (IC_{50})	μg/mL			
R2	Sinapic acid	mg/g dry extract			
R3	Benzoic acid	mg/g dry extract			

*Ethanol/water (w/w).

TABLE 2 | Comparison of experimental data and predictive data obtained by BBD.

	Factors/Levels			**Responses**								
	A	***B***	***C***	**R1 (Lipase inhibition)**			**R2 (Sinapic acid)**			**R3 (Benzoic acid)**		
				Exp.	**Pre.**	**PRE**	**Exp.**	**Pre.**	**PRE**	**Exp.**	**Pre.**	**PRE**
Run	**%**	**°C**	**min**	**(μg/mL)**	**(μg/mL)**	**%**	**(mg/g)**	**(mg/g)**	**%**	**(mg/g)**	**(mg/g)**	**%**
1	−1	1	0	173.808	171.78	−1.18	2.014	2.20	8.74	10.230	10.74	4.75
2	1	0	1	66.227	64.97	−1.93	13.661	13.56	−0.74	19.107	22.81	16.23
3	0	−1	1	74.585	73.80	−1.06	8.209	8.50	3.42	26.672	23.49	−13.55
4	−1	0	−1	153.410	154.66	0.81	6.388	6.49	1.57	26.887	23.19	−15.94
5	1	−1	0	72.486	74.52	2.73	9.748	9.56	−1.97	8.7870	8.27	−6.25
6	0	0	0	73.516	73.51	−0.01	8.257	8.22	−0.45	27.474	26.86	−2.29
7	0	0	0	74.008	73.51	−0.68	8.205	8.22	0.18	26.472	26.86	1.44
8	0	0	0	73.614	73.51	−0.14	8.210	8.22	0.12	26.879	26.86	−0.07
9	1	0	−1	65.201	61.24	−6.47	10.900	11.33	3.80	36.989	33.75	−9.60
10	−1	0	1	116.409	120.37	3.29	6.440	6.01	−7.15	31.890	35.13	9.22
11	0	1	1	102.438	100.51	−1.92	7.363	7.61	3.25	21.777	18.02	−20.85
12	−1	−1	0	146.980	143.80	−2.21	3.363	3.50	3.91	17.942	17.89	−0.29
13	0	0	0	73.413	73.51	0.13	8.208	8.22	0.15	26.586	26.86	1.02
14	1	1	0	89.071	92.25	3.45	8.680	8.54	−1.64	18.543	18.60	0.31
15	0	0	0	73.017	73.51	0.67	8.211	8.22	0.11	26.885	26.86	−0.09
16	0	1	−1	111.156	111.94	0.70	6.754	6.46	−4.55	21.386	24.57	12.96
17	0	−1	−1	91.018	92.94	2.07	8.140	7.90	−3.04	12.178	15.93	23.55

Abbreviations: Exp.: experimental, Pre.: predictive, PRE: percent relative error.

20 μL. A gradient elution program was employed with solvent systems of solution A: 100% methanol and solution B: 2% acetic acid: water (pH 2.65). Measurements were made with the DAD detector in the range of 254–320 nm.

Crude extracts prepared under various conditions were run three times through the HPLC analysis conditions after being diluted with HPLC-grade methanol and filtered through 0.45 μm membranes. The retention times and UV spectra were used for the qualitative determinations of seven different phenolic compounds in extracts. Furthermore, a calibration curve was obtained by running seven phenolic mixture solutions at a range of 5–100 μg/mL with the same HPLC conditions to perform quantitative analysis. The amount of

phenolic compounds in extracts was evaluated by using the calibration curve.

2.4 | Evaluation of Lipase Inhibition

The underlying principle for the lipase inhibition approach is based on the spectroscopic measurement of the absorbance of *p*-nitrophenol molecule obtained from *p*-nitrophenyl butyrate (*p*-NPB) [21]. Our previous modified method was used to evaluate the lipase inhibition of extracts [22].

The experimental procedure involves the coding of microplates labelled *A*, *B*, *C* and *D*. Each microplate is prepared with specific quantities of the following components: *A* consists of 5 μL of buffer solution (0.1 M Tris–HCl buffer, pH 8.0), 90 μL of enzyme solution (crude porcine PL Type II from Sigma, EC 3.1.1.3, at a concentration of 200 units/mL) and 5 μL of substrate solution (10 mM *p*-NPB in acetonitrile); *B* includes 5 μL of buffer solution and 90 μL of enzyme solution; *C* contains 90 μL of enzyme solution, 5 μL of sample solution and 5 μL of substrate solution; *D* is composed of 5 μL of buffer solution, 90 μL of enzyme solution and 5 μL of sample solution. Each microplate was incubated at 37°C for 15 min prior to the addition of the substrate solutions. Following this, the substrate solutions were introduced into the designated wells, and the microplates were incubated again at 37°C for an additional 15 min. The absorbance of the samples was measured at a wavelength of 405 nm, with three replicates conducted for each sample. The absorbance values designated as *A*, *B*, *C* and *D* were used to calculate the levels of lipase enzyme inhibition. The following contains the formula.

$$\text{Lipase inhibition}\,\% = \left[\left[(A-B)-(C-D)\right]/(A-B)\right] * 100$$

Graphs were constructed based on the percentage of enzyme inhibition values obtained at the end of the experiment, plotted against the logarithm of concentration (*y*- and *x*-axes). Subsequently, the IC_{50} values for lipase enzyme inhibition, representing the concentration required for 50% inhibition, were calculated using the equations derived from the graphs.

3 | Results and Discussion

3.1 | HPLC Analysis

The previous method we created was employed for HPLC analysis [20]. The relevant methodology has been mediated to carry out the qualitative evaluation of seven different compounds. Benzoic acid and sinapic acid were identified in all samples' chromatograms of the ultrasonic extraction-prepared extracts. Similarly, benzoic acid and sinapic acid were discovered in extracts prepared with the conventional extraction method (Figure 1).

3.2 | Analysis of Box–Behnken Design

BBD is a response surface methodology used for optimising experimental factors based on three-level factorial designs [23].

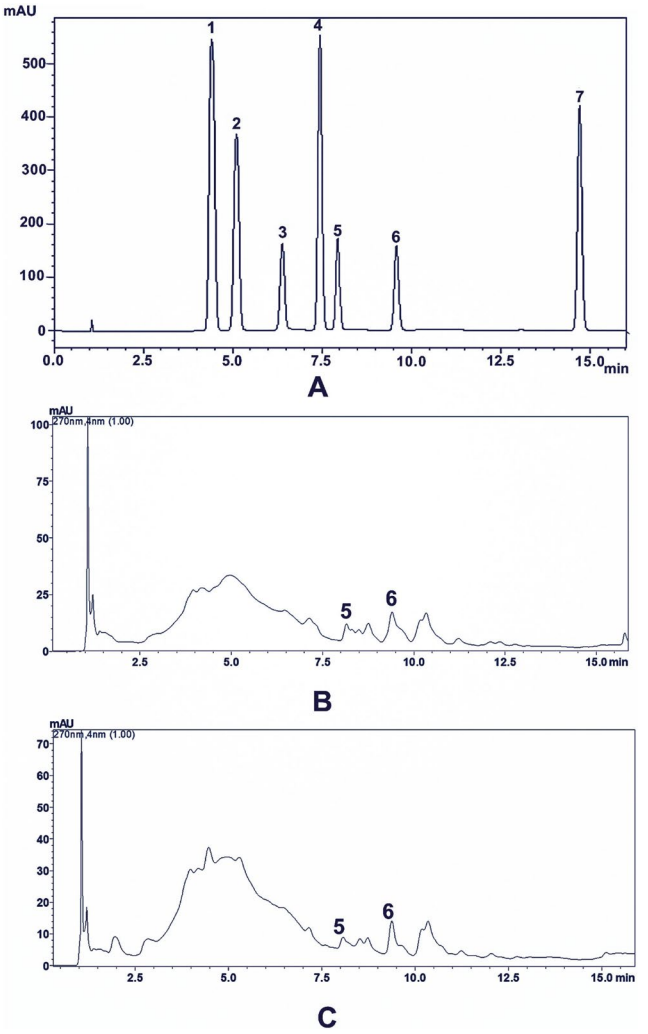


FIGURE 1 | HPLC Chromatograms HPLC chromatogram of phenolic standards, Peak identification: 18. 1: *p*-hydroxybenzoic acid, 2: vanillic acid, 3: syring aldehyde, 4: *P*-coumaric acid, 5: sinapic acid, 6: benzoic acid, 7: quercetin, UV spectrum for sinapic acid (A), HPLC chromatogram of *V. arctostaphylos*' extract prepared by conventional technique, calibration curve and for sinapic acid (B), HPLC chromatogram of *V. arctostaphylos*' extract prepared by ultrasound-assisted extraction at optimal conditions, calibration curve and UV spectrum for sinapic acid (C).

Three levels of independent variables (solvent ratio, temperature and time) were used to achieve the BBD. The BBD technique functions as the clarification of the link between experimental and predictive data [24]. The optimisation becomes more meaningful when the experimental data and the predictive data exhibit similarities. Percent relative error is one of the values used to gauge meaningfulness. It is calculated by the equation as [((predictive data-experimental data)/predictive data)*100] [25]. The BBD experimental design, the experimental data, the predictive data and the value of the percent relative error of all responses are presented in Table 2.

The results demonstrated that there was a meaningful relationship between the predicted and the experimental values of lipase inhibition and sinapic acid because the value of PRE specified was low except for two values. However, the relationship

between the predicted and the experimental values of benzoic acid content was not considered significant (Table 2).

The sequential model sum of squares, model summary, lack of fit tests with quadratic, cubic, two-factor interaction (2FI), and linear models was used for each response to select the optimal model and to obtain the regression equations using ANOVA. Also, the coefficient of variance (CV%), coefficient of determination (R^2) and adjusted coefficient of determination are used to assess the model's dependability (R^2a) [1, 26]. The quadratic model was the most fitting model that combined a significant p value ($p < 0.0001$) as evidenced in the study of the sum of squares and lack of fit test (Table 3).

There are previous studies that have investigated the effects of ethanol concentration, temperature, and time on lipase inhibition using BBD. The previous study on *Morus alba* suggested that the inhibitory effect of lipase inhibition markedly increased with higher concentrations of ethanol. Furthermore, the extraction temperature showed a linear relationship with the inhibitory activity of PL, whereas the extraction time revealed a quadratic impact on this activity [27]. The study on *Ilex paraguariensis* indicated that the linear component of ethanol concentration significantly influenced lipase inhibition, while the interaction two variables, ethanol concentration and extraction time was found to be insignificant [28]. The research concerning *Cudrania tricuspidata* revealed that the linear component of ethanol concentration had the most significant effect on lipase inhibition, whereas the interaction terms between ethanol concentration and extraction time were determined to be insignificant [29].

Table 4 shows the coefficient estimate and p value of two responses. The lipase inhibition was shown to be significantly affected by the effects of the linear coefficients (A, B and C), two quadratic coefficients (A^2 and B^2), and one interactive coefficient (AC) ($p < 0.05$, Table 4). The highest effect for lipase inhibition (IC_{50} value) was based on the ethanol concentration (A) with regression coefficient = −37.40 and significant p value ($p < 0.0001$). The increasing percent ethanol concentration caused the decreasing effect of the IC_{50} value of lipase inhibition. In addition, the temperature (B) had a directly proportional significant effect on the IC_{50} value of lipase inhibition (regression coefficient = 11.43, $p < 0.0001$). Time (C) had an inversely proportional effect (regression coefficient = −7.64, $p = 0.0003$). Also, two quadratic terms (A^2 and B^2) had significant effects ($p < 0.0001$), and both of them had a directly proportional effect (regression coefficient 26.29, 20.78, respectively). An interactive term (AC) had a significant directly proportional effect on the IC_{50} value of lipase inhibition ($p = 0.0006$ and regression coefficient = 9.51). Quadratic terms (C^2) and interactive terms (AB and BC) all had nonsignificant effects due to p values ($p > 0.05$).

Therefore, after eliminating the nonsignificant terms, the regression equation was modified, IC_{50} value of lipase inhibition was the following reduced equation:

$$\text{IC}_{50}\text{ value of lipase inhibition} = 73.51 - 37.20A + 11.43B - 7.64C + 9.51AC + 26.29A^2 + 20.78B^2$$

Consequently, in line with earlier research, a notable correlation was identified between lipase inhibition and ethanol concentration; however, the influence of interactive terms on lipase inhibition was not found to be significant. It was concluded that both extraction time and extraction temperature exerted a greater impact on lipase inhibition than observed in previous studies.

The sinapic acid content was shown to be significantly affected by the effects of the linear coefficients (A, B and C), two quadratic coefficients (B^2 and C^2) and one interactive coefficient (AC) ($p < 0.05$, Table 4). The highest effect for sinapic acid content was based on the solvent ratio (A) with regression coefficient = 3.10 and significant p value ($p < 0.0001$). The increasing percent solvent ratio caused the increasing effect of sinapic acid content. In addition, the temperature (B) had an inversely proportional significant effect on sinapic acid content (regression coefficient = −0.5811, $p = 0.0018$) and time (C) had a directly proportional significant effect (regression coefficient = 0.4364, $p = 0.008$). Also, two quadratic terms (B^2 and C^2) had significant effects ($p < 0.0001$). B^2 had a directly proportional effect (regression coefficient = −2.00), while C^2 had an inversely proportional significant effect on sinapic acid content (regression coefficient = 1.40). An interactive term (AC) had a significant directly proportional effect on the sinapic acid content (p value = 0.005 and regression coefficient = 0.6772). Quadratic terms (A^2) and interactive terms (AB and BC) all had nonsignificant effects due to p values ($p > 0.05$).

Therefore, after eliminating the nonsignificant terms, the regression equation was modified, and sinapic acid content was the following reduced equation:

$$\text{Sinapic acid content} = 8.22 + 3.10A — 0.5811B + 0.4364C + 0.6772AC — 2.00B^2 + 1.40C^2$$

The 3D response surface plots serve as an excellent graphical exhibition of regression equations, showing the interaction between the independent variables and the response [30]. As can be seen in Figure 2, the IC_{50} value of lipase inhibition decreased at the high solvent ratio (ethanol/water [v/v]), while low extraction time. The extraction temperature being between 55°C and 65°C reduced the IC_{50} value at the highest rate, leading to the highest lipase inhibition. Figure 3 exhibits that the sinapic acid content increased when the solvent ratio (ethanol/water [v/v]) and extraction time were high. Similar to the results of lipase inhibition, the extraction temperature between 55°C and 65°C resulted in the maximum concentration of sinapic acid.

Sinapic acid is highly soluble in ethanol but less so in water [31]. This may be the reason for the increase in sinapic acid content as the solvent ratio (ethanol/water [v/v]) increased based on our study. Following the study's findings, it was found that the concentration of sinapic acid increased as the extraction time increased in *Brassica oleracea* L. var. *sabellica*'s ultrasonic extraction at 60°C [32]. Another research indicated that the extraction of sinapic acid from *Fragaria ananassa* is influenced by both temperature and the duration of the extraction process, as demonstrated by the BBD. The optimal temperature for

TABLE 3 | Results of the Box–Behnken design's model adequacy test.

Source	Sum of squares	df	Mean square	*F*	*p*	Remarks
Sequential model sum of squares of lipase inhibition						
Mean vs. total	1564E + 05	1	1.564E + 05			
Linear vs. mean	12583.83	3	4194.61	9.91	0.0011	
2FI vs. Linear	402.62	3	134.21	0.2632	0.8503	
Quadratic vs. 2FI	5026.07	3	1675.36	162.74	< 0.0001	**Suggested**
Cubic vs. quadratic	71.55	3	23.85	186.57	< 0.0001	Aliased
Residual	0.5113	4	0.1278	9.91	0.0011	
Total	1.744E + 05	17	10261.26	0.2632	0.8503	
Sequential model sum of squares of sinapic acid						
Mean vs. total	1036.64	1	1036.64			
Linear vs. mean	81.01	3	27.00	13.01	0.0003	
2FI vs. linear	1.93	3	0.6424	0.2565	0.8550	
Quadratic vs. 2FI	**24.26**	**3**	**8.09**	**71.36**	**< 0.0001**	**Suggested**
Cubic vs. quadratic	0.7912	3	0.2637	554.41	< 0.0001	Aliased
Residual	0.0019	4	0.0005			
Total	1144.62	17	67.33			
Lack of fit tests of lipase inhibition						
Linear	5500.25	9	611.14	4780.79	< 0.0001	
2FI	5097.63	6	849.60	6646.26	< 0.0001	
Quadratic	**71.55**	**3**	**23.85**	**186.57**	**< 0.0001**	**Suggested**
Cubic	0.0000	0				Aliased
Pure Error	0.5113	4	0.1278			
Linear	5500.25	9	611.14	4780.79	< 0.0001	
2FI	5097.63	6	849.60	6646.26	< 0.0001	
Lack of fit tests of sinapic acid						
Linear	26.97	9	3.00	6300.36	< 0.0001	
2FI	25.05	6	4.17	8775.29	< 0.0001	
Quadratic	**0.7912**	**3**	**0.2637**	**554.41**	**< 0.0001**	**Suggested**
Cubic	0.0000	0				Aliased
Pure Error	0.0019	4	0.0005			
Source	**SD**	**R^2**	**Adjusted R^2**	**Predicted R^2**	**PRESS**	**Remarks**
Model summary statistics source of lipase inhibition						
Linear	20.57	0.6958	0.6256	0.4970	9096.87	
2FI	22.58	0.7181	0.5490	0.1074	16142.88	
Quadratic	**3.21**	**0.9960**	**0.9909**	**0.9367**	**1145.61**	**Suggested**
Cubic	0.3575	1.0000	0.9999		*	Aliased
Model summary statistics source of sinapic acid						
Linear	1.44	0.7502	0.6925	0.4846	55.66	
2FI	1.58	0.7680	0.6288	−0.1606	125.33	
Quadratic	**0.3366**	**0.9927**	**0.9832**	**0.8827**	**12.66**	**Suggested**
Cubic	0.0218	1.0000	0.9999		*	Aliased

TABLE 4 | *p* values and coefficient estimate of two responses.

	Lipase inhibition		Sinapic acid	
Source	***p***	**Coefficient estimate**	***p***	**Coefficient estimate**
Model	< 0.0001		< 0.0001	
Intercept		+73.51		+8.22
A-Solvent ratio	< 0.0001	−37.20	< 0.0001	3.10
B-Temperature	< 0.0001	11.43	0.0018	−0.5811
C-Time	0.0003	−7.64	0.0080	0.4364
AB	0.1545	−2.56	0.6889	0.0702
AC	0.0006	9.51	0.0050	0.6772
BC	0.2683	1.93	0.4488	0.1350
A^2	< 0.0001	26.29	0.1462	−0.2681
B^2	< 0.0001	20.78	< 0.0001	−2.00
C^2	0.7560	0.5054	< 0.0001	1.40
Lack of fit test				
Quadratic	< 0.0001		< 0.0001	

TABLE 5 | Predicted and experimental values of optimal conditions and comparison with conventional extraction technique (maceration).

Optimal conditions of the extraction procedure	**Optimise (predicted) values**		**Experimental values**	
Solvent ratio* (100%); temperature (°C) = 60; time (min) = 60	**IC_{50} value of lipase inhibition**	**Sinapic acid content**	**IC_{50} value of lipase inhibition**	**Sinapic acid content**
	64.97 μg/mL	13.56 mg/g	66.22 μg/mL	13.66 mg/g

Conditions of the conventional extraction procedure	**Experimental values**					
Solvent ratio*	**0%**		**50%**		**100%**	
Temperature (°C) = 25; time (min) = 24 h	**IC_{50} value of lipase inhibition**	**Sinapic acid content**	**IC_{50} value of lipase inhibition**	**Sinapic acid content**	**IC_{50} value of lipase inhibition**	**Sinapic acid content**
	169.56 μg/mL	7.26 mg/g	158.32 μg/mL	7.30 mg/g	122.30 μg/mL	7.73 mg/g

*Ethanol/water (w/w).

extraction was determined to be 50.435°C [33]. While this data does not meet the ideal temperature we established, it is relatively close to that benchmark.

Sinapic acid has antiobesity properties lipase inhibition as well as downregulating adipogenesis and lipogenesis process in addition to improving liver and kidney functions in cases of obesity [34]. IC_{50} value of lipase inhibition was determined as 10 μM according to previous research [12]. Additionally, other studies have indicated that in 3T3-L1 adipocytes, sinapic acid promotes mitochondrial biogenesis through the activation of the AMPK, p38 MAPK and CREB signalling pathways, resulting in the browning of white adipocytes [35]. It was demonstrated that the intervention with dietary sinapic acid effectively inhibited weight gain and decreased fatty tissue mass in obese hamsters. The administration of sinapic acid resulted in a downregulation of triglycerides, total cholesterol, nonesterified fatty acids (NEFA) and low-density lipoprotein cholesterol levels, while simultaneously increasing high-density lipoprotein cholesterol levels and fecal TC content [36].

As mentioned above, both sinapic acid content and lipase inhibition increased with increasing solvent ratio and temperature of 55°C–65°C. The effect on lipase inhibition may be directly related to the increment of the antiobesity-effective compound, sinapic acid.

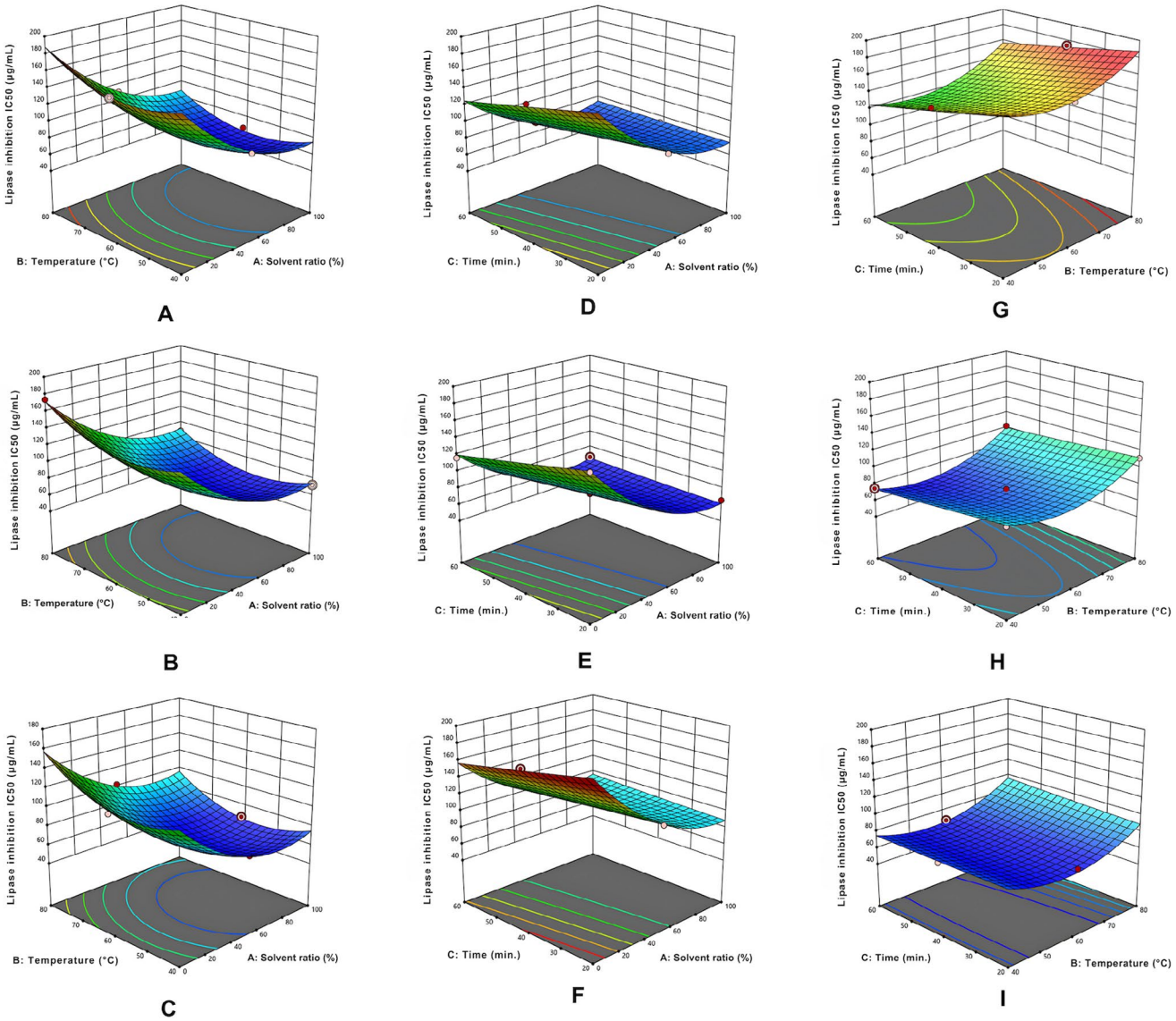


FIGURE 2 | 3D response surface plots for IC_{50} value of lipase inhibition. Effect of temperature, the solvent ratio for IC_{50} value of lipase inhibition and their relationship with different extraction times 20 min (A), 40 min (B), 60 min (C); Effect of time, the solvent ratio for IC_{50} value of lipase inhibition and their relationship with different temperatures 40°C (D), 60°C (E) and 80°C (F); effect of time, temperature for IC_{50} value of lipase inhibition and their relationship with solvent ratio 0% (G), 50% (H) and 100% (I).

3.3 | Accuracy of Box–Behnken Design

It is necessary to validate the fitted model to ensure adequate approximation to the actual values [26]. The accuracy of the Box–Behnken design was evaluated by comparing it with diagnostic plots of the experimental and designed model. Diagnostic plots of the experimental and predicted values in Figure 4A,B showed the model adequacy and the correlation between the experimental and predicted values for lipase inhibition and sinapic acid. The diagnostic plots of the experimental and model in these graphs were observed to localise close to the straight line. So, there was sufficient fit between the experimental data and the model data. The results showed that the Box–Behnken model is selective for lipase inhibition and determination of sinapic acid concentration.

3.4 | Optimisation of Extraction Procedures and Comparison With Conventional Extraction Technique (Maceration)

The desirability score is an important value to assess optimal conditions. The closeness of the desirability score to 1 increases the accuracy of the conditions [37]. The model proposed optimal conditions related to minimising response desirability for lipase inhibition and maximising response desirability for sinapic acid content to formalise the model equation. Desirability scores were evaluated as 1 for lipase inhibition, 0.9912 for sinapic acid content and 0.4700 for benzoic acid content (Figure 5). Also combined desirability was found as 0.8583. The optimal conditions to supply the highest sinapic acid content and the highest lipase inhibition effect were 100% of solvent ratio (ethanol/water

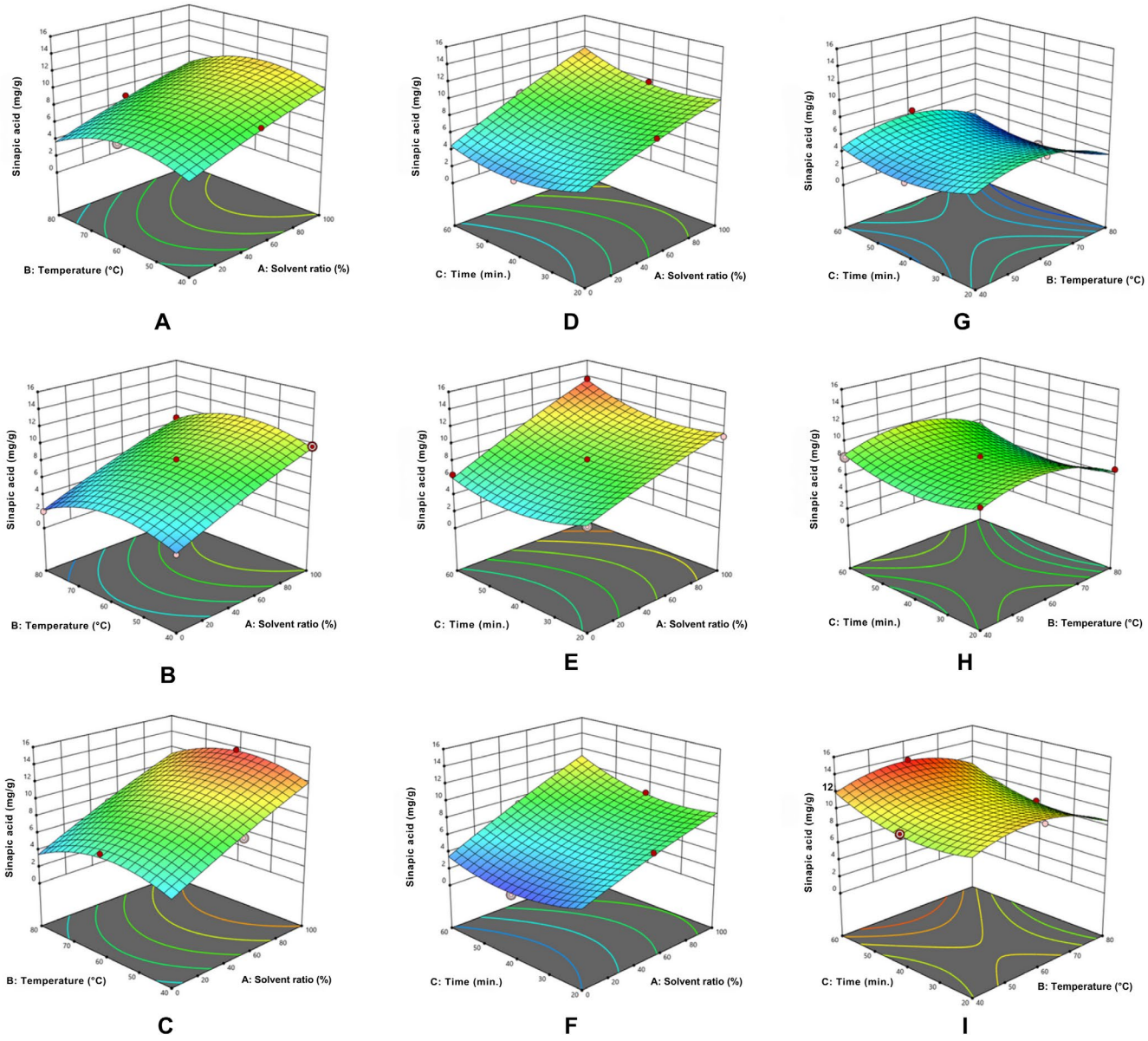


FIGURE 3 | 3D response surface plots for sinapic acid content. Effect of temperature, the solvent ratio for IC_{50} value of sinapic acid content and their relationship with different extraction times 20 min (A), 40 min (B), 60 min (C); Effect of time, the solvent ratio for sinapic acid content and their relationship with different temperatures 40°C (D), 60°C (E) and 80°C (F); Effect of time, temperature for sinapic acid content and their relationship with solvent ratio 0% (G), 50% (H) and 100% (I).

[v/v]), extraction temperature of 60°C and extraction time of 60 min (Table 5).

It was observed that the sinapic acid content and the lipase inhibitory effect increased as the solvent ratio increased for the classical extraction technique. The green extraction method in optimal conditions significantly outperformed the conventional extraction method using the same solvent ratio in terms of sinapic acid concentration and lipase inhibitory effect. Optimum conditions caused a remarkable increase of 57.01% for sinapic acid content and a significant increment of 53.12% for lipase inhibitory effect compared with the classical extraction technique (Table 5). Thus, the optimum conditions of the ultrasonic green extraction technique, which mediates the extraction with a shorter time, less energy consumption, high efficiency for sinapic acid content and lipase inhibition, were determined for Caucasian whortleberry.

The effect of Caucasian whortleberries on obesity and obesity-related disorders has been the subject of numerous studies. In a triple therapy for overweight/obese hypertensive patients, the hydroalcoholic extract of the *V. arctostaphylos* berry and two common antihypertensive medications reduced systolic and diastolic blood pressure without adverse interactions and adverse effects with other antihypertensive drugs [38]. It was demonstrated that the hydroalcoholic extract of *V. arctostaphylos* significantly fruit decreased both blood fasting and postprandial glucose, and also HbA1c levels in 37 patients with Type 2 diabetes, with no discernible effects on liver or kidney function [39]. *V. arctostaphylos*' hydroalcoholic fruit extract

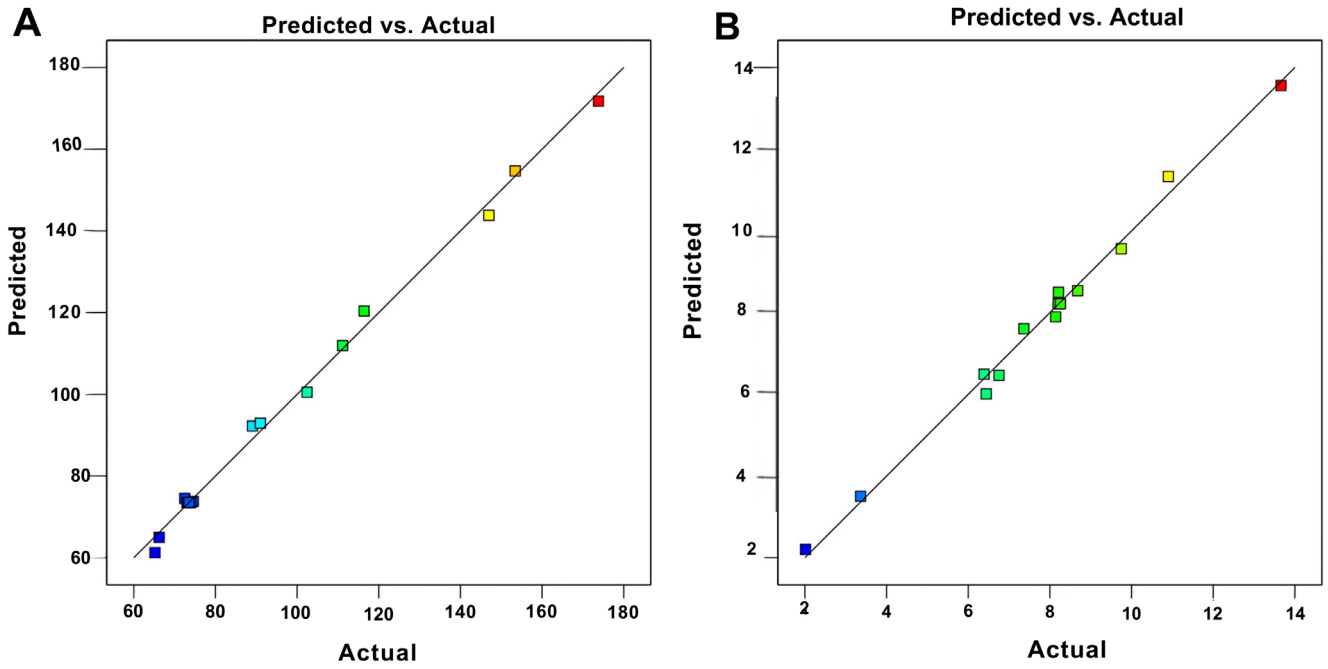


FIGURE 4 | Diagnostic plots for the Box–Behnken model accuracy of predicted and actual data against lipase inhibition (A) and sinapic acid content (B).

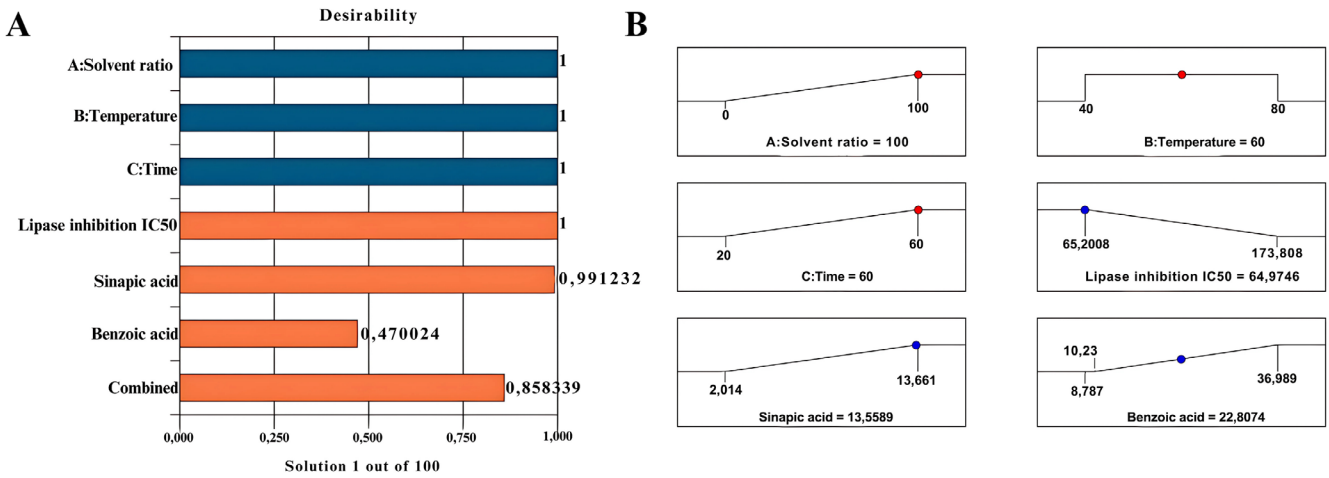


FIGURE 5 | Desirability bar and ramp for optimising extraction conditions.

has been proven to have therapeutic effects on blood lipid profile and oxidative stress in 61 hyperlipidemic adult patients [40]. The findings of a study proved that *V. arctostaphylos* fruit ethanolic extract is a potent antidiabetic agent, with antihyperglycemic, antioxidant and triglyceride-lowering effects on alloxan-diabetic Wistar rats. It has been shown that this antihyperglycemic effect may be associated with the inhibition of α-glucosidase activity and upregulation of INS and GLUT-4 gene expression [4]. To the best of our knowledge, although there are many studies on *V. arctostaphylos* for obesity-related disorders, no study has been found on the lipase inhibitory effect of the species.

The optimal conditions of the green ultrasonic extraction of Caucasian whortleberry in terms of sinapic acid content and lipase inhibition determined by this study can create more efficient, more eco-friendly and effective results for obesity-related further research.

4 | Conclusion

Our findings showed that the Box–Behknen model and the equations generated from the model are reliable and can be applied to predict the optimal condition of ultrasonic extraction for the enhancement of the sinapic acid content and lipase inhibitory effect for Caucasian whortleberry. 100% solvent ratio (ethanol/water [v/v]), extraction temperature of 60°C and extraction time of 60 min were shown to be the optimum conditions for both lipase inhibition and sinapic acid content. Utilisation of the green ultrasonic extraction technique mediated to obtain extracts with higher efficiency and effectiveness

and reduced the extraction time and energy consumption compared with the classical maceration technique. These conditions can be suggested for the design and production of pharmaceutical forms including Caucasian whortleberry extract enhanced with sinapic acid and lipase inhibitory effect, which may be employed in obesity and obesity-related disorders.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

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