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# Ultrasound-Assisted Phytochemical Extraction Condition Optimization Using Response Surface Methodology from Perlette Grapes (*Vitis vinifera*)

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**Abstract:** In the current study, bioactive compounds of *Vitis vinifera* (Perlette) were extracted using an ultrasound-assisted extraction technique. The central composite design of response surface methodology (RSM) was used to determine the effect of time, temperature, and concentration of acetic acid on response variables that include extract yield, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of *Vitis vinifera* extracts. The results of the central composite design of RSM revealed that the quadratic polynomial model is best fitted to experimental results, with all the responses having a regression coefficient greater than 0.9. Optimized extraction levels include 26.5 min, an extraction temperature of 59 °C, and an acetic acid concentration of 62.9% with good extraction yield results of 34.95 g/100 g dry weight (DW) of grapes, TPC 34.38 mg gallic acid equivalent per gram (GAE/g) DW, flavonoid content 10.21 mg quercetin equivalents per gram (QE/g) DW, and antioxidant activity of 9.11 mg/100 mL ascorbic acid equivalent. The present study showed that acetic acid can be effectively used for the ultrasound-assisted extraction of polyphenols, flavonoids, and antioxidants of *Vitis vinifera*. These optimized conditions can be used for the extraction of bioactive compounds that can be for the development of nutraceutical products.

**Keywords:** antioxidants; flavonoids; phytochemicals; ultrasound; *Vitis vinifera*

## 1. Introduction

Grapes (*Vitis vinifera*) are widely grown throughout the world and mainly used for wine making. Grapes are a rich source of plant secondary metabolites such as polyphenols, flavonoids, and anthocyanins, etc. [1]. Due to the presence of these phytochemicals, grapes and grape products possess antioxidant properties that are beneficial for health. In addition to nutritional significance, grapes also possess medicinal and health-promoting properties [2–4].

For the utilization of polyphenols and flavonoids, it is necessary to isolate and identify these compounds. For the separation and identification of flavonoids, one of the critical and most important step is the extraction of these compounds from the food matrix. Several techniques have been developed to improve the extraction of plant secondary metabolites, which include enzymatic extraction [5], supercritical fluid extraction [6], microwave-assisted extraction [7], soxhlet extraction [8], and ultrasound-assisted extraction [9]. Ultrasonic extraction in comparison with other methods of extraction is much more efficient, using less solvent and greater time-saving techniques for phytochemicals extraction [10]. There are several factors that influence the extraction of phytochemicals from the food matrix, which include the solvent used for extraction, as well as the time and temperature

of extraction [11]. Furthermore, the process used for extraction is also important, it can reduce the cost of extraction along with time [12]. In the past, several extraction techniques were used, which include Soxhlet extraction, maceration, and stirring. However, now it is time to use that method of extraction that has the maximum recovery of these compounds with the least damage/destruction of bioactive compounds, lowest cost, and least time required for extraction [3]. The present study was designed to extract polyphenols from grapes using ultrasonic techniques, and extraction conditions were optimized for the time of extraction, temperature of extraction, and solvent concentration using response surface methodology. In the past, several studies have reported the extraction capabilities of ethanol and methanol, but limited studies are available that describe the efficiency of acetic acid for the extraction of bioactive compounds. The present study investigates the ultrasound-assisted extraction potential of acetic acid and optimizes the extraction conditions to obtain the maximum yield of polyphenols from grapes.

## 2. Materials and Methods

Samples of grapes were collected from the Al Awan grapes (Attock) farm and Pothowar grapes farm (Kallar Sayedan, Rawalpindi) at a fully mature stage. The precooling of samples was done using ice, and samples were transported to the Institute of Food and Nutritional Sciences, PMAS Arid Agriculture University Rawalpindi, Punjab, Pakistan. The washing and cleaning of samples was done to remove dust and other foreign material from samples; after that, blanching was carried out at 55–60 °C for 5 min. Samples were oven dried to constant weight under vacuum and ground to powder form, packed in air-tight glass bottles, and stored for further analysis.

A powdered grape sample of 2 g was placed in a volumetric flask and extraction solvent was added to make the volume 100 mL. Magnetic stirring was done for 5 min to homogenize the mixture. Extraction was carried out on a sonicator (230VAC, Cole-Parmer, Vernon Hills, IL USA) at a frequency of 40 kHz, and power was set at 250 W. The time and temperature of sonication varied at different levels, as described in Table 1.

A central composite rotatable design with three variables at five different levels was used to optimize the extraction conditions for extraction yield, total phenolic content, total flavonoid content, and antioxidant activity from Perlette grapes. In this study, the variables studied include time of extraction, temperature of extraction, and concentration of solvent.

**Table 1.** Coded and actual levels of independent variables used in response surface methodology.

Variables	Coded Level				
	$-\alpha$	-1	0	+1	$+\alpha$
	Experimental Actual Values				
Extraction time (X1)	11.6	15	20	25	28.4
Temperature (X2)	33.2	40	50	60	66.8
Acetic acid Conc. (X3)	33.2	40	50	60	66.8

### 2.1. Extract Yield Determination

The filtered extract 10 mL was concentrated using a rotary evaporator to remove the solvent. The concentrated extract was dried in a vacuum oven until constant weight, and the result is represented as g/100 g dry basis.

### 2.2. Determination of Phenolics

Spectroscopic techniques utilizing Follin–Ciocalteu's reagent was used to determine the total phenolic content of Perlette grapes extract. A sample diluted extract of 1 mL was mixed with 9 mL of deionized water. Sodium carbonate (7%) was added to the mixture after 5 min. The mixture was mixed and diluted to the volume of 25 mL. After that, the mixture was incubated for 80 min at room temperature. Different concentration of gallic acid solutions were prepared and used as

standard solution to construct a calibration curve by measuring absorbance at 750 nm using a spectrophotometer. Samples were also analyzed on spectrophotometer (UV-9200 Biotech Engineering Management Co., Ltd., Nicosia, Cyprus, UK) at 750 nm, and the results are presented as gallic acid equivalent per gram (GAE/g).

### 2.3. Determination of Flavonoids

Flavonoid content was determined by following the method of Singleton et al. [13]. Briefly, 0.3 mL of extracts, 3.4 mL of 30% methanol, 0.15 mL of 0.5 M.  $\text{NaNO}_2$ , and 0.15 mL of 0.3 M  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  was mixed in a 10-mL test tube. After 5 min, 1 mL of 1 M NaOH was added. The mixture was measured at 506 nm in a spectrophotometer (UV-9200 Biotech Engineering Management Co., Ltd., Nicosia, Cyprus, UK). The standard curve was prepared using different concentrations of quercetin (0–300 mg/L). The total flavonoids are expressed as milligrams of quercetin equivalents per gram (QE/g) dry matter of grapes.

### 2.4. 2,2-Diphenyl-2-picrylhydrazyl (DPPH) Assay

The antioxidant assay of grapes was done by using a relatively stable radical 2,2-diphenyl-2-picrylhydrazyl radical (DPPH) by adopting the method of Brands-Williams et al. [14]. DPPH stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol. The working solution was prepared by diluting stock solution to obtain the absorbance of  $1 \pm 0.02$  using the spectrophotometer. Forty microliters of extract were mixed with 3 mL of DPPH working solution and kept in the dark for 30 min at room temperature. The absorbance was measured at 515 nm using a spectrophotometer. The antiradical activity was determined based on the decrease in absorbance by using the following equation:

$$\text{ARA}(\%) = \frac{(\text{Control absorbance} - \text{sample absorbance})}{(\text{Control absorbance})} \times 100$$

### 2.5. Experimental Design and Statistical Analysis

To investigate the effect of independent variables related to time (X1), temperature (X2), and acetic acid concentration (X3) on extract yield, total phenolic content, total flavonoid content, and antioxidant activity, response surface methodology using a central composite design with four central points was used. The coded and uncoded levels of independent variables that were used in the design are presented in Table 1. Actual values of independent variables were calculated by the following equation:

$$X = (X_0 - X_C)/\Delta X$$

where X and  $X_0$  represent coded and actual values of independent factors, respectively.  $\Delta X$  indicates a change in step, and  $X_C$  represents the values at a central point. Central points used for time (min), temperature ( $^{\circ}\text{C}$ ), and acetic acid concentration (%) include 20%, 50%, and 50%, respectively, while the step change for time is 5, and 10 for temperature and acetic acid as well. The analysis of variance technique was used to calculate the RSM statistical parameters.

Data was analyzed by Design Expert software 7.0.0 for building the model, calculation of the predicted values, and three-dimensional graphs plotting for depicting the effect of independent variables on the response variables. Regression equations were calculated for the best-fitted model, having lack of fit non-significant with pure error. The Fisher test value (F-value), coefficient of determination  $R^2$ , and lack of fit were used to determine the quality and adequacy of the model.

## 3. Results and Discussion

For optimization of the process for total phenolic content, extract yield, flavonoid content, and antioxidant activity for Perlette grapes using ultrasound-assisted extraction at different levels of solvent

concentration, time and temperature of extraction central composite design was built, which is shown in Table 2. The total phenolic content, flavonoid content, extract yield, and antioxidant activity at different experimental conditions are also shown in Table 2. The model is statistically significant with a non-significant lack of fit as compared to noise with adequate values of  $R^2$  for the model to be used. The statistical parameters calculated by ANOVA techniques are presented in Table 3, while the mean square values for the model, independent variables, interaction of independent variables, and for lack of fit are presented in Table 4.

Different factors affecting the efficiency of ultrasound-assisted extraction include the time of extraction, temperature of extraction, and concentration of solvent. In order to optimize the extraction conditions, a three-factor, five-level, central composite design of response surface methodology is used [15]. The factors used in the experiment include the time of extraction, which varied between 12 and 28 min, temperature of extraction, which was from 33 to 67 °C, and concentration of acetic acid, which ranged from 33% to 67%. Experimental data were used to compute the coefficient of the polynomial equation that predicts response values. Regression equations calculated by response surface methodology for various responses are presented below:

$$\text{Yield} = +33.75 + 1.19A - 3.53B - 0.16C - 1.84AB + 1.42AC - 0.081BC + 0.86B^2 - 1.26B^2 + 1.5C^2$$

$$\text{TPC} = +24.28 + 1.71A + 1.11B + 0.27C + 4.46AB + 3.13AC + 0.22BC - 1.04A^2 - 0.37B^2 - 1.30C^2$$

$$\text{TFC} = +11.08 - 0.74A + 1.03B + 1.37C + 3AB - 1.09AC - 0.35BC - 1.19A^2 - 0.54B^2 - 0.85C^2$$

$$\text{ARA} = +8.5 + 1.342A + 1.35B + 1.12C + 0.86AB - 0.19AC + 0.77BC - 1.36A^2 - 1.38B^2 - 1.24C^2$$

Analysis of variance showed that the experimental results could be well fitted with a quadratic model polynomial with coefficients of determination ( $R^2$ ) for yield, TPC, TFC, and antioxidant activity being 0.927, 0.919, 0.889, and 0.960, respectively. The coefficient of determination and other statistical parameters calculated from ANOVA are presented in Table 3.

The quadratic model was found to be statistically significant with a non-significant lack of fit ( $p < 0.05$ ) with respect to pure error, which showed that the model is statistically accurate. The  $R^2$  values closer to unity are an indication of better fitting the model to experimental data, and contrary if the values of  $R^2$  are lower, which shows that the independent variables are not predicting the values of responses accurately [16].

**Table 2.** Three-factor, five-level central composite design used for the extraction of total phenolic content (TPC), total flavonoids content (TFC), and antioxidant activity of extracts. GAE/g: gallic acid equivalent per gram, QEQ/g: quercetin equivalents per gram.

Runs	Time (X1) (min)	Temperature (X2) (°C)	Solvent Concentration (X3) (%)	Yield (g/100 g)	TPC mg GAE/g	TFC (mg QEQ/g)	DPPH (%)
1	0 (20)	0 (50)	0 (50)	32.05	22.92	10.18	8.72
2	1 (25)	1 (60)	1 (60)	30.475	34.39	11.84	9.404
3	-1 (15)	-1 (40)	1 (60)	35.2	20.55	14.15	2.35
4	-1 (15)	1 (60)	-1 (40)	33.35	19.12	4.76	0.73
5	0 (20)	0 (50)	0 (50)	34.625	25.64	9.87	8.03
6	1 (25)	1 (60)	-1 (40)	28.1	25.78	12.98	6.5
7	0 (20)	1.68 (67)	0 (50)	25.7	24.79	11.21	7.64
8	-1 (15)	-1 (40)	-1 (40)	38.175	25.32	9.51	1.79
9	0 (20)	0 (50)	0 (50)	33.75	24.92	12.54	8.24
10	1.68 (28)	0 (50)	0 (50)	40	21.37	4.76	6.85
11	-1.68 (12)	0 (50)	0 (50)	32.4	19.92	8.9	3.45
12	0 (20)	0 (50)	0 (50)	34.55	23.87	12.03	8.83
13	0 (20)	0 (50)	-1.68 (33)	38.775	19.63	5.32	3.32
14	0 (20)	-1.68 (33)	0 (50)	34.675	20.31	6.15	2.54
15	0 (20)	0 (50)	1.68 (67)	37.25	20.19	10.26	7.68
16	1 (25)	-1 (40)	1 (60)	43	21.896	6.01	3.94
17	-1 (15)	1 (60)	-1 (40)	30.85	12.77	9.65	4.93
18	1 (25)	-1 (40)	-1 (40)	39.5	16.62	4.06	3.58

**Table 3.** Statistical parameters of central composite rotatable design of response surface methodology (RSM).

Model Term	Extract Yield	TPC	TFC	Antioxidant Activity
Model ( <i>p</i> -value)	0.0011	0.0017	0.005	0.001
Lack of Fit ( <i>p</i> -value)	0.22	0.17	0.37	0.07
R <sup>2</sup>	0.92	0.91	0.88	0.95

**Table 4.** Statistical result of response surface methodology.

Source	df	Mean Squares			
		Yield	TPC	TFC	Antioxidant Activity
Model	9	33.76 ***	36.06 ***	17.01 ***	14.23 ***
X1-Time (Min)	1	19.41 *	39.98 **	7.51 *	27.38 **
X2-Temperature (°C)	1	170.07 ***	16.95 *	14.36 **	25.03 **
X3-Conc. (%)	1	7.4 *	22.25 *	25.47 **	17.24 **
X1X2	1	27.20 **	158.8 *	72.0 ***	5.88 *
X1X3	1	16.10 *	78.17 ***	9.51 *	0.27 NS
X2X3	1	0.05 NS	0.38 NS	1.0 NS	4.79 *
X1 <sup>2</sup>	1	9.38 *	13.74 *	17.96 **	23.53 **
X2 <sup>2</sup>	1	20.22 **	1.72 NS	3.67 NS	24.23 **
X3 <sup>2</sup>	1	28.54 **	21.42 *	9.20 *	19.45 **
Residual	8	2.95	3.55	2.39	0.66
Lack of Fit	5	3.86 NS	4.83 NS	2.76 NS	0.97 NS
Pure Error	3	1.43	1.41	1.77	0.14

\* Significant at  $p < 0.1$ , \*\* Significant at  $p < 0.05$ , \*\*\* Significant at  $p < 0.001$ , NS Non significant.

### 3.1. Effect of Independent Variables on Extract Yield

The yield of extract depends on the time of extraction due to its significant effect on extract yield at a linear ( $p < 0.001$ ) and interaction level ( $p < 0.05$ ) with the concentration of solvent. Other factors that affect the yield of extract include a linear term of temperature ( $p < 0.001$ ), quadratic effect of temperature ( $p < 0.05$ ), concentration of acetic acid ( $p < 0.05$ ), and interaction terms of time and concentration of solvent ( $p < 0.05$ ).

The effect of time and temperature of extraction on the extract yield is depicted in Figure 1a. The time of extraction has a linear effect on yield, while the temperature of extraction has a quadratic effect on the yield of the extract. The concentration of solvent and temperature of extraction has a quadratic effect on the yield of extract. A similar trend was observed by Ghafoor et al. [17], who optimized the ultrasonic condition for grape seed extract. Similar effects was also observed by Lapornik et al. [18], who extracted from red grapes.

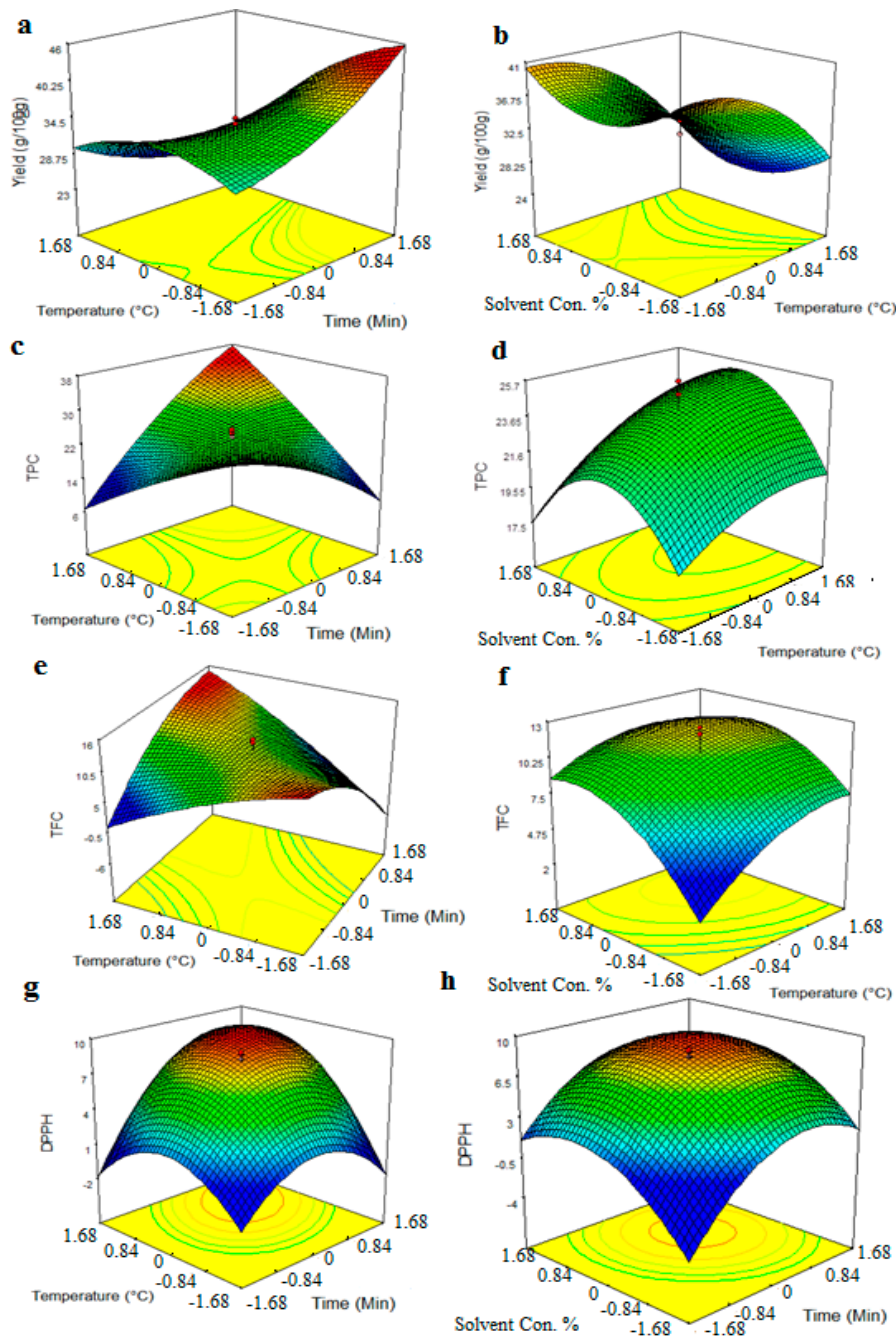
### 3.2. Effect of Factors on Total Phenolic Content

The total phenolic content of extract was measured using Follin–Ciocalteu reagent, which depends upon the phenolic compounds' oxidation and phosphomolybdic–phosphotungstic acid. The total phenolic content of the extract is shown in Table 2. Total phenolic compounds are reported as gallic acid equivalent and ranged from 12.77 to 34.39 mg GAE/g; the highest content of phenolic content was found in the second run, having a time of extraction, temperature of extraction, and concentration of acetic acid 25 min, 60 °C, and 60% acetic acid respectively, while minimum phenolic compounds were found in run 17 at 15 min, 60 °C, and 40% for the time, temperature, and concentration of acetic acid for extraction, respectively.

The analysis of variance table shows that the time of extraction has a maximum impact on total phenol extraction followed by the temperature and concentration of acetic acid. A higher concentration



of acetic acid will increase the content of hydrophobic compounds, while a low concentration of acetic acid extract contains a higher amount of water-soluble compounds. Figure 1c shows the effect of time and temperature of extraction on the total phenolic content of extract while keeping the concentration of solvent constant (50%). This effect of time is due to the increasing contact time of solvent with the extraction material to improve the diffusion of compounds [19,20].



**Figure 1.** Response surface graph for the optimization of (a) extract yield (g/100 g) versus time (min) and temperature (°C) of extraction; (b) extract yield (g/100 g) versus temperature (°C) and solvent concentration (%); (c) TPC mg GAE/g versus time (min) and temperature (°C) of extraction (d) TPC mg GAE/g versus temperature (°C) and solvent concentration (%); (e) TFC (QE/g) versus time (min) and temperature (°C) of extraction; (f) TFC (QE/g) versus temperature (°C) and solvent concentration (%); (g) Antioxidant activity versus time (min) and temperature (°C) of extraction; (h) Antioxidant activity versus time (min) and solvent concentration (%).

The total phenolic content of extract increases with the increase of time of extraction, but after some time, the TPC content decreases, which is due to the destruction of phenolic compounds exposed to higher temperatures for a longer period of time [17,21]. Similarly, a quadratic effect has been observed for the concentration of solvent and temperature of extraction while keeping the temperature constant (Figure 1d). Increasing the concentration of acetic acid increased the TPC content of the extract, but after a certain limit, the TPC content starts decreasing, because increasing the acetic acid concentration above a certain limit decreases the water content of the solvent, while water plays an important role in the extraction of hydrophilic phenolic compounds.

### 3.3. Effect of Factors on Total Flavonoid Content

Analysis of variance shows that the quadratic model is statistically significant for the total flavonoid content of extract with a non-significant lack of fit with respect to pure error. Figure 1e shows the effect of different factors on the total flavonoid content of extract, keeping some factors at a constant level. The maximum flavonoid content was found in the extract of run no. 12 (12.453 mg QEQ/g), as shown in Table 2, while the minimum flavonoid content was extracted in run 18 (4.06 mg QEQ/g), in which the time of extraction was 25 min with 40 °C and 40% acetic acid concentration. The solvent concentration and temperature of extraction has a quadratic effect on the total flavonoid content of extract, keeping the time of extraction constant. The time of extraction has a linear effect, while the temperature has a quadratic effect on the total flavonoid content. Keeping the concentration of acetic acid constant, as shown in Figure 1f, increasing the concentration of solvent relative to time increases the TFC content of the extract, keeping the temperature constant. This might be due to the presence of flavonoid compounds that are more soluble in organic solvent as compared to water. Similar results were also reported byGhafoor et al. [22], who optimized the extraction conditions using ethanol as solvent for the extraction of total anthocyanins with the assistance of ultrasound technique. The effect of time of extraction and temperature of extraction is shown in Figure 1e; there is a quadratic effect of time of extraction and temperature on the total flavonoid content of extract. With the increasing temperature of extraction, the TFC content increases to some extent, but starts decreasing by a certain amount due to the destruction of compounds at high temperatures [21,23].

### 3.4. Effect of Factors on Antioxidant Activity

The antioxidant activity of extract using different levels of independent variables is shown in Table 2. The highest antioxidant activity (9.40 mg/mL) was found in run 2 with 25 min time of extraction, 60 °C temperature, and 60% acetic acid concentration. The antioxidant activity of extract is linearly dependent with time ( $p < 0.001$ ), temperature ( $p < 0.001$ ), concentration of solvent ( $p < 0.001$ ), interaction of time and temperature ( $p < 0.05$ ), and the time and concentration of the solvent. Similarly, a quadratic effect was also significant ( $p < 0.05$ ) for time, temperature, and concentration of solvent.

Three-dimensional plots showing the effect of independent variables are presented in Figure 1g. The interactive effect of time of extraction and temperature of extraction is quadratic. With the increase in the time of extraction and temperature of extraction, the antioxidant activity increases gradually up to some extent, and then starts decreasing. High temperatures destroy the bioactive compounds having antioxidant activity, so the longer these compounds are exposed to high temperatures, the greater the loss of phytochemicals. A similar trend was observed by [17], who extracted grape seed polyphenols using ethanol as the solvent. The findings of the present study are in line with respect to the response variables with the change in independent variables, but antioxidant activity was found to be less i.e., 10.17 mg/mL as compared to 12.02 mg/mL. This is due to polyphenols that mostly accumulate in the skin and seeds of grapes, while the present study was carried out on a seedless grape variety. The present study showed that acetic acid can also be used efficiently for the extraction of phytochemicals from grapes with good antioxidant activity.

### 3.5. Independent Variable Optimization

To demonstrate the effect of time, temperature, and concentration of acetic acid, three-dimensional graphs were drawn using design expert software (Design Expert 7.0.0). In these graphs, two variables were varied in experimental ranges, and the third variable was kept constant at a central point. Figure 1a,c,e,g was drawn by varying the time and temperature of extraction, while using a concentration of solvent at a central point (50%). Figure 1b,d,f,g was plotted by changing the temperature and concentration of solvent, keeping the time of extraction at a central point (20 min). These plots demonstrated complex interactions among time, temperature, and concentration of solvent.

Using design expert software, numeric optimizations of responses were carried out by the desirability function. Optimization was carried out with the goal set to maximize the extract yield, total phenolic content, total flavonoid content, and maximum antioxidant activity using the independent variables of time, temperature, and concentration of solvent in the ranges that were used in the experiment. Four different solutions were calculated by design expert software with different levels of independent variables with good desirability. The solution with maximum desirability had a selected time of 26.5 min, temperature 59 °C, and 63% concentration of solvent. Similarly, the aqueous optimized extraction time for grape pomace using ultrasound-assisted extraction was found to be 25 min by [24]. The results of the present study are much better than the findings of [25], who optimized the extraction condition for phytochemicals from grape by-products by response surface methodology having a time of extraction of 93 min, temperature 94 °C, and 60% aqueous ethanol concentration. These findings are comparable with the results of [22], with the time of extraction being 24.5 min, a temperature of 45.14 °C, and 52.35% ethanol concentration. Similar results were also reported by [9], who optimized the extraction conditions for total phenolics using ultrasound-assisted extraction by response surface methodology from blue berry wine pomace. The optimized extraction conditions include a 23.67 min time of extraction and 61.03 °C temperature. The predicted and actual values of responses at optimized conditions are shown in Table 5. The results of experimental values are in well agreement with the response values that are predicted. In comparison, the optimal condition for microwave-assisted extraction includes a 30-min time of extraction and 150 mL/g solvent to the sample ratio [26].

**Table 5.** Experimental and predicted values of responses at optimized conditions of independent variables.

Optimized Condition	Coded Levels	Real Values
Time (min)	1.3	26.5
Temperature (°C)	0.9	59
Solvent Concentration (%)	1.29	62.9
Response	Experimental Values	Predicted Values
Yield (g/100 g)	33.84 ± 1.8	34.95
TPC mg (GAE/g)	35.12 ± 2.3	34.38
TFC (mg QEQ/g)	10.05 ± 0.76	10.21
Antioxidant activity mg/100 mL	8.78 ± 0.91	9.11

## 4. Conclusions

In the present study, ultrasound-assisted extraction of phytochemicals from *Vitis vinifera* (Perlette) grapes was carried out using different levels of time, temperature, and concentrations of acetic acid, and the extraction condition was optimized using a central composite design of RSM. The present study demonstrates that response surface methodology is a useful technique for the optimization of independent variables for phytochemical extraction such as phenolics and flavonoids. The findings showed that the independent variables of time, temperature, and solvent concentration have a significant impact on responses with a quadratic model of central composite design with a lack of fit



non-significant at  $p < 0.05$  and regression coefficients values closer to unity. Furthermore, the present study showed that acetic acid can also be used as a medium of extraction for phytochemicals.

**Author Contributions:** M.K.: Design of study, execution of experiment, statistical analysis, write-up, A.A.: Design of study, execution of experiment, write-up, R.M.A.: Design of study, experiment, write-up, G.K.R.: Design of study, experiment, write-up.

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