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Antilipidemic, antidiabetic, and antioxidant activities of Isabella grape pomace extract: A design of experiments approach

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Abstract

Food processing by-products are considered valuable sources of bioactive compounds with applications in the food, pharmaceutical, cosmetic, and natural colorant industries. The present study aimed to investigate the phytochemical composition and the pancreatic lipase, cholesterol esterase, and α-glucosidase inhibitory activities of Isabella grape (*Vitis labrusca*) pomace.

Extracts were prepared from Isabella grape pomace using a design of experiments approach. The phenolic content of the extracts was analyzed by RP-HPLC, and the effects of extraction parameters were evaluated. Ethanol concentration, microwave power, and extraction temperature were identified as the most influential factors. A Box-Behnken design was employed to optimize the extraction conditions. The optimized extract was further evaluated for its antioxidant and enzyme inhibitory activities. The results demonstrated that the optimized extract exhibited inhibitory effects against pancreatic lipase, cholesterol esterase, and α-glucosidase. In addition, strong antioxidant activity was confirmed using CUPRAC, FRAP, and DPPH radical scavenging assays. These findings indicate that Isabella grape pomace could be considered a promising natural source of bioactive compounds with antioxidant and metabolic enzyme inhibitory potential. Therefore, grape processing by-products may contribute to the development of functional ingredients and nutraceutical candidates targeting oxidative stress and metabolic disorders such as obesity and diabetes.

Keywords: *Vitis labrusca*, lipase, cholesterol esterase, α-glucosidase, Box-Behnken

1. Introduction

The valorization of food processing by-products has attracted increasing interest in recent years due to their low cost, environmental benefits, and potential health-promoting properties. These by-products can be used as valuable sources of bioactive compounds in various sectors, including food, pharmaceutical, cosmetic, and natural colorant industries [1,2]. Grape pomace, which consists primarily of skins, seeds, and stems remaining after processing, has emerged as a promising raw material due to its high content of oils, proteins, pectins, sugars, and phenolic compounds. Consequently, this agro-industrial by-product can be recycled as animal feed, fertilizer, food additives, and functional ingredients, contributing to both economic and environmental sustainability [3].

V. labrusca L. (Isabella grape), widely cultivated in the Black Sea Region of Türkiye, is distinguished by its characteristic aroma and flavor. The fruit is traditionally consumed fresh and processed into various products such as juice, marmalade, molasses, vinegar, and pickles [4–8]. Previous phytochemical studies have reported that *V. labrusca* is rich in phenolic compounds, organic acids, vitamins, minerals, terpenes, and other bioactive constituents that may contribute to its nutritional and therapeutic value [4,8,9]. Grapes and grape-derived products are particularly recognized for their high phenolic content, including anthocyanins, proanthocyanidins, flavonols, catechins, and resveratrol. These compounds not only contribute to the sensory characteristics of grapes but are also associated with a

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wide range of biological activities. Literature indicates that the extracts obtained from grapes have anticarcinogenic, antibacterial, antifungal, antiviral, antioxidant, and antiedema properties, as well as potential for wound healing, inflammation, diabetic retinopathy, atherosclerosis, microcirculatory disorders, night blindness, and capillary fragility including antioxidant, antimicrobial, anti-inflammatory, and cardioprotective effects [5,7,10]. In addition, grape pomace has attracted attention as a source of natural pigments and functional ingredients for food and industrial applications [3,11–14].

Dietary phenolic compounds have also been reported to contribute to weight management and metabolic health through multiple mechanisms, including inhibition of digestive enzymes involved in lipid and carbohydrate metabolism, suppression of adipocyte differentiation, enhancement of thermogenesis, and modulation of appetite regulation [15–18]. In particular, inhibition of pancreatic lipase, cholesterol esterase, and α-glucosidase has emerged as an important strategy for reducing lipid absorption and postprandial hyperglycemia, thereby supporting the prevention and management of obesity and type 2 diabetes.

Optimization of extraction processes plays an essential role in maximizing the recovery of bioactive compounds from natural resources. Owing to its efficiency and reliability, Response Surface Methodology (RSM) is a widely used statistical application that enables the simultaneous evaluation of multiple process variables while minimizing the number of experimental runs required [19–21].

Although numerous studies have investigated the phytochemical composition and biological activities of grapes and grape processing by-products, information regarding the metabolic enzyme inhibitory properties of Isabella grape pomace remains limited. In particular, no study has comprehensively evaluated the pancreatic lipase and cholesterol esterase inhibitory activities of pomace obtained from Isabella grapes cultivated in the Eastern Black Sea Region of Türkiye. Therefore, the present study aimed to optimize the extraction of bioactive compounds from Isabella grape pomace using Response Surface Methodology and to characterize the phenolic composition of the resulting extracts. Furthermore, the optimized extract was evaluated for its antioxidant capacity and its inhibitory activities against pancreatic lipase, cholesterol esterase, and α-glucosidase. To the best of our knowledge, this is the first study to investigate these biological activities in Isabella grape pomace from this region using a design of experiments approach, thereby providing new insights into the valorization of grape-processing by-products as potential functional ingredients for metabolic health applications.

2. Materials and methods

2.1. Chemicals

The chemical used in biological activity studies, ethanol, methanol, hydrochloric acid (HCl), methanol-HPLC grade, acetic acid, Folin-Ciocalteu's phenol reagent, DPPH, gallic acid, sodium carbonate, trolox, BHT, tris, sodium acetate (CH_3COONa), potassium chloride (KCl), TPTZ, iron (III) chloride ($FeCl_3$), copper (II) chloride dihydrate ($CuCl^2.2H_2O$), neocuproine, *p*-nitro phenyl butyrate (*p*-NPB), orlistat, 4-Nitrophenyl β-d-glucuronide (PNPG), glutathione, acarbose, α-glucosidase (from *Saccharomyces cerevisiae*, EC 3.2.1.20, ≥100 units/mg protein), and pancreatic lipase enzyme (Crude porcine PL type II, EC 3.1.1.3, 200 U/mL), were purchased from Sigma-Aldrich.

In this study, a magnetic stirrer (Heidolph MR), refrigerator (Arçelik), evaporator (Heidolph), precision balance (Ohaus Pioneer), incubator (Memmert), pH meter (Hanna), centrifuge (Elektromag), spectrophotometer (BMG Labtech Spectrostar Nano), vortex (Heidolph Reax Top Vortex Mixer), freeze dryer (Christ), microwave oven (START SYNTH), ultrasonic water bath (Isolab), and HPLC (Shimadzu) were used.

2.2. Plant material and extraction methods

Grape-processing by-products remaining after grape processing, boiling, and juice filtration were obtained from a local commercial company (Trabzon, Türkiye) and stored at -80 °C until lyophilization. The pomace was lyophilized and milled prior to extraction.

2.2.1. Experimental design and extraction optimization

Fractional factorial design and Box-Behnken design were implemented using Design-Expert 11.1.2 software (Stat-Ease Inc., USA) to optimize extraction conditions [22]. Experimental design was performed using Design Expert 11.1.2 software based on a two-level fractional factorial design with six independent variables: extraction time, temperature, ethanol concentration, microwave power, ultrasonic time, and acid ratio. The extracts were prepared according to the extraction conditions defined by the minimum and maximum values of the independent variables. 1.5 g portion of lyophilized pomace was extracted with 20 mL of solvent based on Table 1.

The phenolic contents of the 32 extracts prepared according to the fractional factorial design were analyzed by HPLC. Vanillic acid was selected as the response variable for the optimization because it was consistently detected in all extracts and exhibited a well-defined chromatographic peak, allowing its reliable quantification and the monitoring of changes in

Table 1. Experimental design conditions

	Independent Variables					
	Microwave time (s)	Temp. (°C)	Acid ratio (%)	Microwave power (W)	Ultrasonic time (s)	Ethanol conc. (%)
1	120	25	0.01	500	60	0
2	60	50	0.01	500	60	0
3	60	50	0.001	500	20	0
4	120	50	0.001	300	20	0
5	120	50	0.01	500	20	0
6	120	25	0.001	500	20	0
7	60	50	0.001	500	60	70
8	120	25	0.001	300	20	70
9	120	25	0.001	500	60	70
10	120	25	0.01	300	20	0
11	120	50	0.001	300	60	70
12	120	25	0.001	300	60	0
13	60	50	0.001	300	20	70
14	60	50	0.01	500	20	70
15	60	50	0.01	300	20	0
16	60	25	0.001	500	20	70
17	60	50	0.01	300	60	70
18	120	50	0.01	300	60	0
19	60	25	0.001	300	60	70
20	60	25	0.01	300	20	70
21	120	50	0.01	500	60	70
22	60	50	0.001	300	60	0
23	120	50	0.001	500	20	70
24	60	25	0.01	300	60	0
25	60	25	0.001	500	60	0
26	120	50	0.01	300	20	70
27	60	25	0.001	300	20	0
28	60	25	0.01	500	60	70
29	120	50	0.001	500	60	0
30	60	25	0.01	500	20	0
31	120	25	0.01	500	20	70
32	120	25	0.01	300	60	70

Temp.: Temperature, conc.: Concentration

extraction efficiency. In addition, its biological significance regarding enzymes involved in carbohydrate and lipid metabolism has been reported in previous studies [23–32]. Based on the results of this screening design, temperature, ethanol concentration, and microwave power were determined as the most influential factors affecting extraction efficiency. Subsequently, a Box-Behnken design was employed to optimize these three variables. Fifteen additional extracts were prepared in random order with three replicates at the center point for these three parameters (Table 2), and then the phenolic contents of these extracts were also analyzed. The experimental data were subjected to multiple regression analysis, and the model was statistically evaluated using analysis of variance (ANOVA).

2.3. HPLC analysis

The contents of benzoic acid, quercetin, syringaldehyde, *p*-hydroxybenzoic acid, *p*-coumaric acid, sinapic acid, and vanillic acid in extracts prepared based on the fractional factorial design and Box-Behnken design conditions were analyzed using the RP-HPLC method described by Korkmaz et al. (2019) [33]. A mixed stock solution containing each standard at a concentration of 100 µg/mL was prepared in HPLC-grade methanol. Working standard solutions at concentrations of 5, 10, 25, 50, and 100 µg/mL were prepared by appropriate dilution of the stock solution and used to construct the calibration curves. All standard solutions were filtered through 0.45 µm membrane filters before injection. The analyses were carried out using a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) equipped with an LC-20AT pump, DGU-20A5 degasser, SIL-20A autosampler, CTO-10ASVP column oven, and SPD-M20A diode-array detector. An RP-C18 column (150 × 4.6 mm, 5 µm) maintained at 25 °C was used for chromatographic separation. The mobile phase consisted of 100% methanol (solvent A) and 2% (v/v) aqueous acetic acid (pH 2.7; solvent B). The gradient elution program was set as follows (time, %A): 0.01 min, 20%; 4.00 min, 30%; 7.00-8.00 min, 40%; 10.00-11.00 min, 45%; 12.00-13.00 min, 50%; 14.00 min, 60%; and 15.30 min, 80%. Solvent B was adjusted accordingly to maintain a total mobile-phase composition of 100%. The flow rate was 1.5 mL/min, the injection volume was 20 µL, and chromatograms were recorded at 270 nm.

Table 2. Box-Behnken optimization parameters

	Temperature (°C)	Ethanol concentration	Microwave power (W)
1	37.5	0	500
2	50.0	0	400
3	25.0	35	500
4	25.0	0	400
5	25.0	35	300
6	50.0	35	500
7	37.5	35	400
8	37.5	35	400
9	25.0	70	400
10	37.5	35	400
11	50.0	35	300
12	37.5	0	300
13	37.5	70	500
14	37.5	70	300
15	50.0	70	500

2.4. Total phenolic and monomeric anthocyanin content analysis

Total phenolic and monomeric anthocyanin contents of extracts prepared based on the fractional factorial design conditions were determined spectrophotometrically. The total soluble phenolic content of the extracts was determined using the method described by Slinkard and Singleton (1977) [34]. Extracts (10 mg/mL) and standard solutions (gallic acid; 1000-15.63 µg/mL) were mixed with distilled water and Folin-Ciocalteu reagent, incubated at room temperature for 3 min, and then treated with 20% Na_2CO_3 solution. The mixtures were further incubated at room temperature for 2 h, after which the absorbance values of the samples were measured at 765 nm using a spectrophotometer. A

calibration curve was constructed from the absorbance values of gallic acid solutions, and the total phenolic content of the extracts was expressed as mg GAE/g dry extract. All experiments were performed in triplicate, and blanks were prepared using the corresponding solvents of the samples.

The anthocyanin content of the extracts was determined using the pH differential method with modifications [35]. Briefly, 6.25 mL of 1% HCl solution was added to 100 mg of dry extract, mixed and centrifuged for 15 min. The resulting supernatant was divided into two portions, and 0.2 mL of each portion was diluted to 2 mL with pH 1.0 KCl-HCl buffer and pH 4.5 CH_3COONa-HCl buffer, respectively. After equilibration for 30 min, the absorbance of each sample was measured at both 520 and 700 nm. The monomeric anthocyanin content of the samples was calculated as cyanidin-3-glucoside equivalents using the following equation and subsequently normalized to the dry extract amount. The final results were expressed as mg cyanidin-3-glucoside equivalents/g dry extract.

$$\text{Total monomeric anthocyanin content } (\frac{\text{mg}}{\text{L}}) = \frac{(\text{A x Ma x SF})}{(\varepsilon \text{ x l})} \times 1000$$

A= pH 1.0 ($A_{520\,nm} - A_{700\,nm}$) – pH 4.5 ($A_{520\,nm} - A_{700\,nm}$);

M_a (cyanidin-3-glucoside, cyn-3-glucoside): 449.2 g/mol;

SF: dilution factor;

ε: 26900 L x mol^{-1} x cm^{-1};

l: the cell path length, cm.

2.5. Antioxidant activity studies

The antioxidant capacity of the optimized extract was evaluated using FRAP, CUPRAC, and DPPH radical scavenging assays.

FRAP and CUPRAC assays were performed to evaluate the antioxidant power of the optimized extract from species via maceration and ultrasonic-assisted microwave extraction. The assays are based on measurement of the Fe^{3+} and Cu^{2+} reducing capacities of the extracts. Trolox (62.5-1000 µM) was used as a standard and the Fe^{3+}/Cu^{2+} reducing capacities of the extracts were calculated from the Trolox calibration curve, subsequently normalized to the amount of dry extract used in the assay and stated as µmol Trolox equivalents (TE)/g dry extract [36,37]. The extracts were dissolved at a concentration of 10 mg/mL.

In the DPPH radical scavenging assay, appropriate concentrations of the sample were determined by preliminary tests, and then dilutions were prepared. BHT (0.01 mg/mL) was used as the standard. In the experiment, 750 µL of sample and 750 µL of DPPH solution (100 µM) were mixed, vortexed, and incubated for 55 minutes at room temperature in the dark. The absorbance values of the samples were then measured at 517 nm. Solvent was used as a blank, and all experiments were performed in triplicate. The SC_{50} values were calculated from the logarithmic plots of concentration versus absorbance [38].

2.6. Enzyme inhibition studies

The antilipidemic and antidiabetic activities of the optimized extract were evaluated by evaluating pancreatic lipase, cholesterol esterase, and α-glucosidase inhibitory effects.

The pancreatic lipase inhibitory activity of the optimized extract was measured using a modified spectrophotometric method [39]. The extract (12.5-2000 µg/mL) and lipase enzyme were dissolved in Tris-HCl buffer (pH 8, 0.1 mM). p-NPB (10 mM) in acetonitrile was used as a substrate solution. Orlistat (12.5-200 µg/mL) was used as the positive control. After the sample and enzyme solution were added to the wells, the plates were incubated at 37 °C for 15 minutes. At the end of incubation, substrate solution was added and incubated again at 37 °C for 10 minutes. The lipase inhibition was determined by measuring the absorbance at 405 nm.

Cholesterol esterase inhibition of the optimized extract was determined using a method described by Zor et al. (2022) [40]. The sample (5-160 µg/mL), cholesterol esterase enzyme (1 µg/mL), and taurochoic acid (12 mM) were dissolved in phosphate buffer (0.1 M NaCl, pH 7). p-NPB (10 mM) was used as substrate. Simvastatin was used as a positive control. The samples, taurocholic acid solution, buffer, and substrate solution were added to the wells and incubated at 37 °C for 10 minutes. At the end of incubation, enzyme solution was added to all wells and incubated at 37 °C for 25-30 minutes. The cholesterol inhibition was determined by measuring the absorbance at 405 nm.

The α-glucosidase inhibitory activity was determined using a modified method [41]. The samples (125-2000 µg/mL), α-glucosidase enzyme (0.4 U/mL), and glutathione (1 mM) were dissolved in phosphate buffer (pH 6.8). The PNPG solution (3 mM) was used as a substrate. Acarbose (125-2000 µg/mL) was used as a positive control. The sample, glutathione, α-glucosidase, buffer, and substrate solution were added to the wells and incubated at 37 °C for 15 minutes. The reaction was stopped by adding sodium carbonate (0.2 M). α-glucosidase inhibition was determined by measuring the absorbance at 400 nm. The inhibition rate was calculated using the formula:

Inhibition rate (%) = [(ΔAcontrol − ΔAsample)/ ΔAcontrol] × 100.

The half-maximal inhibitory concentrations (IC_{50}) were calculated from the graphs plotted with the logarithmic concentrations of the extract versus inhibition rate (%).

2.7. Statistical analyses

In the *in vitro* studies, each sample was analyzed in triplicate. In the enzyme inhibition assays, IC_{50} values were calculated from plots of percentage inhibition against the logarithm of sample concentration using Microsoft Excel. Experimental results were given as means ± standard deviation (SD). Statistical differences were assessed using one-way ANOVA, followed by Tukey's multiple comparisons test, by GraphPad Prism 8.0.2 (GraphPad Prism Software, San Diego, CA, USA).

3. Results and discussion

The experimental design power obtained with Design Expert 11.1.2 is expected to be a statistical power above 80%. The statistical power of the design was 98.3%, as seen in Fig. 1.

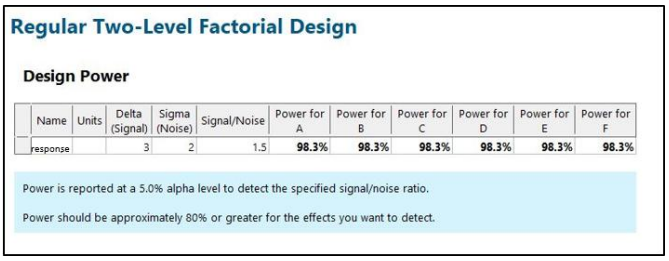

Figure 1. The experimental design power

The phenolic contents of the extracts were examined using HPLC analysis. Among the detected compounds, vanillic acid was selected as the marker compound for optimization studies due to its observation in all extracts and its exhibition of a well-defined chromatographic peak.

The most influential factors affecting extraction efficiency were identified as ethanol percentage, microwave power, and temperature. Similarly, Moutinho et al. (2023) [42] reported that solvent composition, acidification, and temperature substantially influenced the recovery of phenolic compounds from grape pomace. Hydroethanolic solvents facilitate the extraction of compounds with different polarities, while water promotes matrix swelling and solvent penetration. Elevated temperature improves compound solubility and mass transfer, whereas acidic conditions may enhance the stability of acid-sensitive phenolic constituents. These physicochemical effects collectively contribute to the improved extraction efficiency achieved under optimized conditions. The Pareto chart shows the relative significance of these factors and their contributions to the extraction process (Fig. 2). The high F-values and low p-values obtained from ANOVA showed that the models used in the present study were statistically significant (Table 3 and Table 4). The interaction effects of the selected variables on the vanillic acid response are presented in Fig. 2, which shows the regions associated with minimum and maximum response values.

Table 3. Fractional factorial design ANOVA data

Source	Sum of Squares	Mean Square	F Value	*p*-value	
Model	732.69	66.61	4.86	0.0011	Significant
A:Microwave time	13.57	13.57	0.9896	0.3317	
B:Temperature (°C)	34.89	34.89	2.54	0.1264	
C: Acid ratio	22.54	22.54	1.64	0.2145	
D: Microwave power (W)	37.52	37.52	2.74	0.1137	
E: Ultrasonic time (s)	6.25	6.25	0.4555	0.5075	
F: Ethanol concentration	455.33	455.33	33.20	< 0.0001	
EF	42.25	42.25	3.08	0.0945	
ACD	39.73	39.73	2.90	0.1043	
Residual	274.31	13.72			
Cor. Total	1007.00				

Table 4. Box-Behnken ANOVA quadratic model data

Source	**Sum of Squares**	**Mean Square**	**F Value**	***p*-value**	
Model	354.76	39.42	37.22	0.0005	Significant
A: Ethanol concentration	138.53	138.53	130.80	< 0.0001	
B: Microwave power (W)	24.41	24.41	23.04	0.0049	
C:Temperature (°C)	39.38	39.38	37.18	0.0017	
AB	3.25	3.25	3.06	0.1404	
AC	33.00	33.00	31.16	0.0025	
BC	36.32	36.32	34.29	0.0021	
A^2	34.16	34.16	32.25	0.0024	
B^2	49.55	49.55	46.79	0.0010	
C^2	0.2477	0.2477	0.2339	0.6491	
Residual	5.30	1.06			
Lack of Fit	4.90	1.63	8.30	0.1095	Not significant
Pure Error	0.3939	0.1970			
Cor. Total	360.06				
R^2	0.9727	CV %	5.08	Std. deviation	3.66

In this study, *p*-hydroxybenzoic acid, vanillic acid, syringaldehyde, *p*-coumaric acid, sinapic acid, benzoic acid, and quercetin contents of extracts were analyzed using RP-HPLC. The chromatograms obtained from RP-HPLC analysis of standards and optimized extract are given in Fig. 3.

At the same time, the total phenolic and anthocyanoside contents of the extracts were analyzed, and the results of the analyses are shown in Fig. 4 and Table 5.

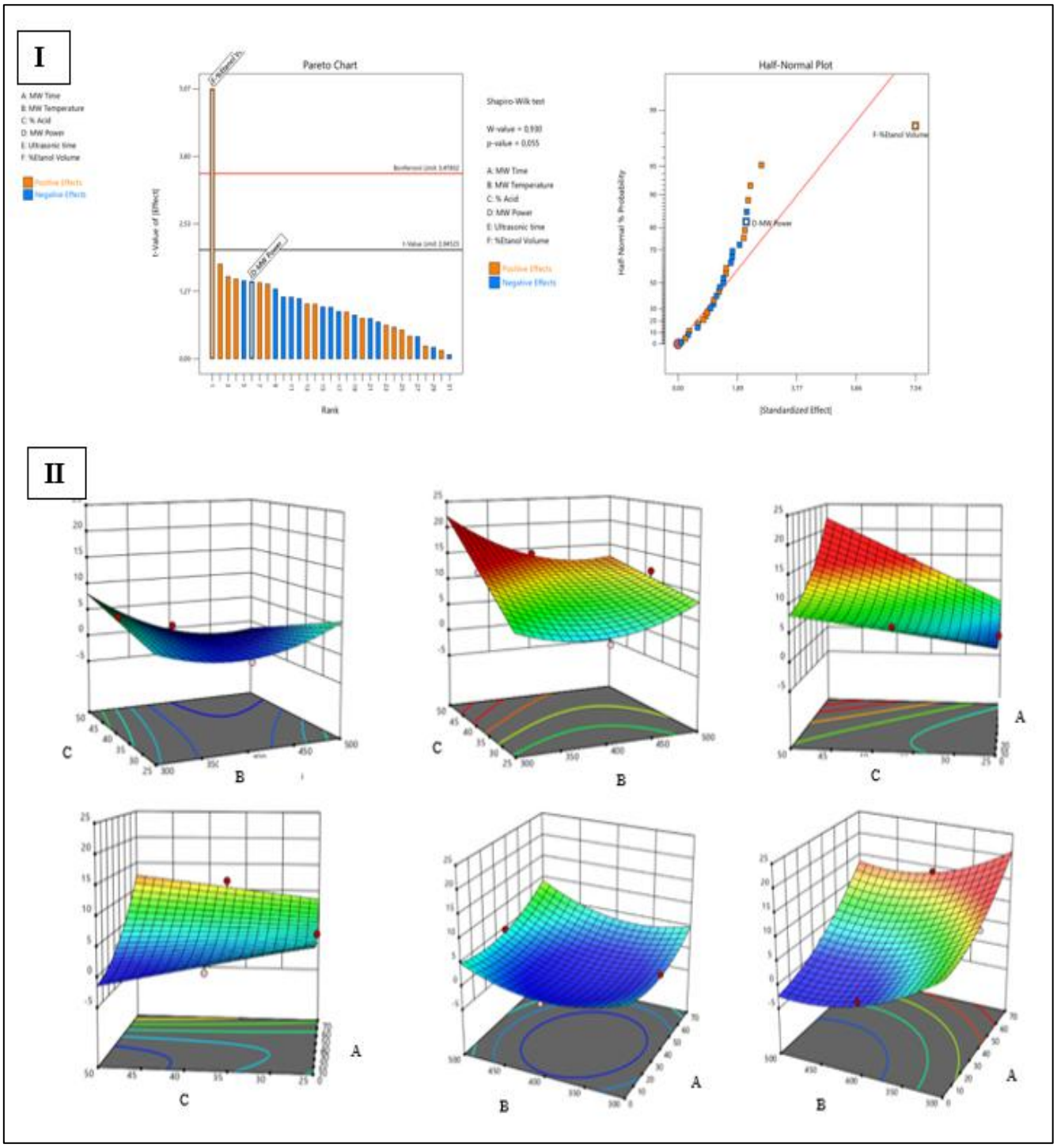

Figure 3. Pareto chart (I) and the minimum and maximum effects of the interactions on the vanillic acid (II)

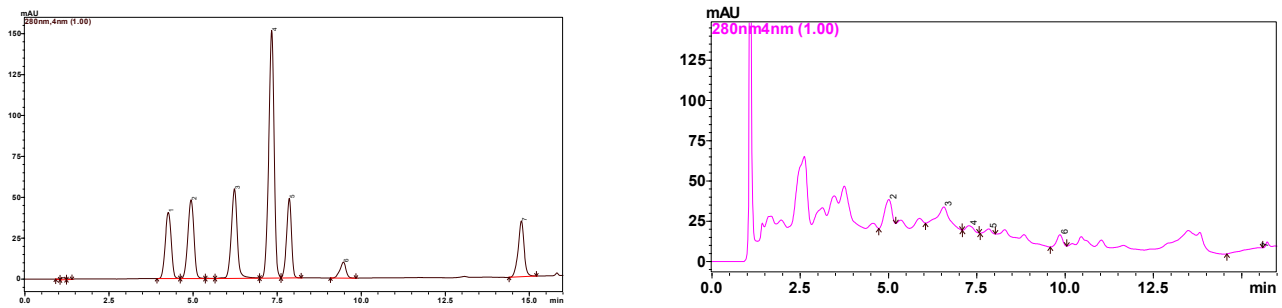

Figure 2. The chromatograms standard phenolic compounds (A), the optimized extract (B) Peak identification: (1) *p*-hydroxy benzoic acid, (2) vanillic acid, (3) syringaldehyde, (4) *p*-coumaric acid, (5) sinapic acid, (6) benzoic acid, (7) quercetin

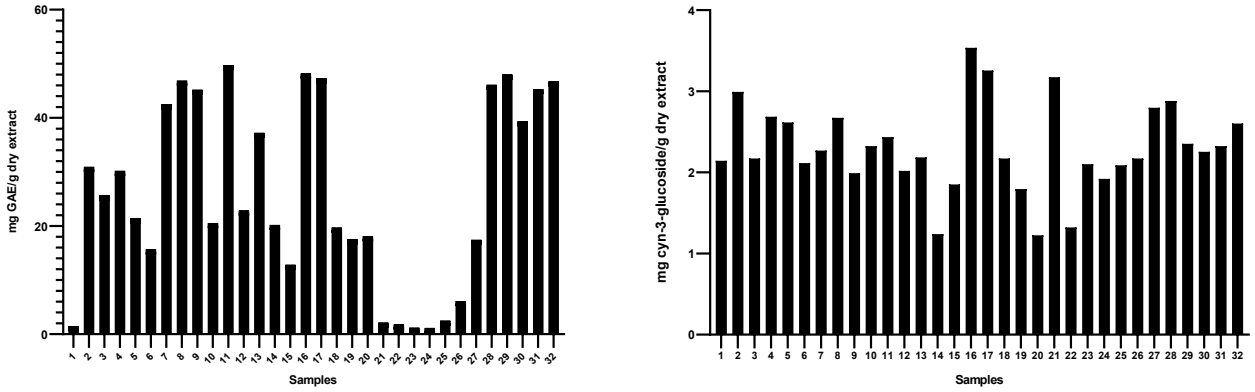

Figure 4. Total phenolic and monomeric anthocyanin contents of extracts prepared based on the fractional factorial design conditions

Table 5. Total phenolic and monomeric anthocyanin contents of extracts

Samples	TPC GAE (mg/g dry extract)	TAC (mg cyn-3-glucoside /g dry extract)
1	1.483 ± 0.167	2.143 ± 0.096
2	30.928 ± 0.347	2.992 ± 0.188
3	25.678 ± 0.174	2.171 ± 0.191
4	30.177 ± 0.241	2.686 ± 0.024
5	21.427 ± 0.293	2.616 ± 0.096
6	15.650 ± 0.301	2.115 ± 0.087
7	42.511 ± 0.567	2.268 ± 0.650
8	46.844 ± 0.297	2.672 ± 0.221
9	45.178 ± 0.210	1.990 ± 0.064
10	20.539 ± 0.127	2.324 ± 0.245
11	49.733 ± 0.144	3.256 ± 0.324
12	22.844 ± 0.962	2.018 ± 0.147
13	37.206 ± 0.337	2.185 ± 0.105
14	20.178 ± 0.293	1.239 ± 0.134
15	12.817 ± 0.289	1.851 ± 0.296
16	48.178 ± 0.241	3.535 ± 0.278
17	47.289 ± 0.376	2.435 ± 0.232
18	19.678 ± 0.962	2.171 ± 0.042
19	17.539 ± 0.174	1.795 ± 0.110
20	18.094 ± 0.699	1.225 ± 0.814
21	2.122 ± 0.376	3.173 ± 0.695
22	1.789 ± 0.567	1.322 ± 0.939
23	1.178 ± 0.255	2.101 ± 0.556
24	1.122 ± 0.127	1.920 ± 0.167
25	2.483 ± 0.250	2.087 ± 0.072
26	6.0944 ± 0.419	2.171 ± 0.221
27	17.428 ± 0.104	2.797 ± 0.326
28	46.094 ± 0.835	2.881 ± 0.543
29	47.983 ± 0.712	2.352 ± 0.272
30	39.344 ± 0.293	2.254 ± 0.261
31	45.317 ± 0.947	2.324 ± 0.401
32	46.733 ± 0.167	2.602 ± 0.450

Data are presented as mean ± SD. $p < 0.0001$, Statistical differences among samples were analyzed by ordinary one-way ANOVA followed by Tukey's multiple comparisons test.

When the obtained TPC and TAC values were evaluated in relation to the extraction conditions, it was observed that the extracts prepared under conditions 11 and 16 in the table include the highest phenolic and monomeric anthocyanin contents. Condition 11 (microwave time 120 s, temperature 50 °C, acid ratio 0.001, microwave power 300 W, ultrasound time 60 s, ethanol concentration 70%) yielded 49.733 ± 0.144 mg GAE/g dry extract and 3.256 ± 0.324 mg cyn-3-glucoside/g dry extract. Similarly, condition 16 (microwave time 60 s, temperature 25 °C, acid ratio 0.001, microwave power 500 W, ultrasound time 20 s, ethanol concentration 70%) resulted in 48.178 ± 0.241 mg GAE/g dry extract and 3.535 ± 0.278 mg cyn-3-glucoside/g dry extract. These findings indicate that extraction parameters significantly affect both phenolic and anthocyanin yields. In particular, changes in extraction temperature and microwave time appear to play a decisive role in enhancing the recovery of bioactive compounds.

Based on the combined evaluation of RP-HPLC analysis, TPC, and TAC results, antioxidant, antilipidemic, and antidiabetic effects of the extract prepared under the optimized conditions were investigated. The results obtained from antioxidant capacity assays are shown in Table 6. The FRAP and CUPRAC values of the optimized extract were expressed as μmol Trolox equivalents (TE)/g dry extract using the Trolox calibration curve. The calibration graphs for Trolox are given in Fig. 5.

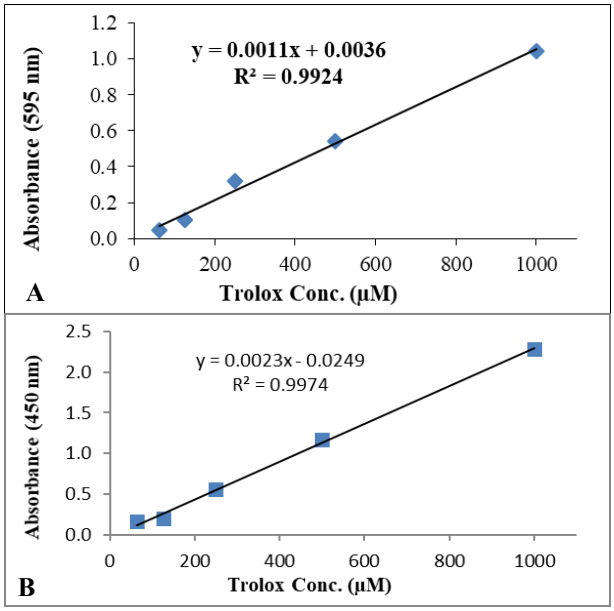

Figure 5. The calibration graphs of trolox used in FRAP (A) and CUPRAC (B) determination

Table 6. The antioxidant capacity of the optimized extract

Samples	CUPRAC[1]	FRAP[2]	DPPH[3]
Optimized extract	98.155 ± 0.262	71.279 ± 0.187	0.0569 ± 0.0006
BHT	-	-	0.0055 ± 0.0004

[1]CUPRAC value indicates copper reducing antioxidant power (μmol TE/g dry extract); [2]FRAP value indicates iron-reducing antioxidant power (μmol TE/g dry extract); [3]DPPH value shows DPPH radical scavenging activity (SC_{50} mg/mL)

The DPPH radical scavenging activity of the optimized extract was compared to the effect of BHT. The graphs obtained from the concentration-absorbance values of the samples are shown in Fig. 6.

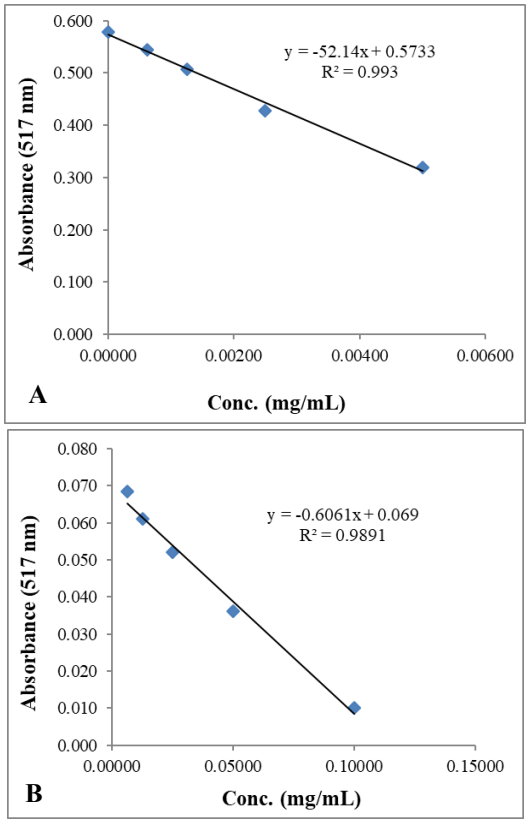

Figure 6. The calibration graphs of BHT (A) and the optimized extract (B)

The antilipidemic and antidiabetic effects of the optimized extract were determined via pancreatic lipase, cholesterol esterase, and α-glucosidase inhibitions. The results are presented in Table 7, Fig. 7–Fig. 9.

Table 7. The antilipidemic and antidiabetic effects of the optimized extract

Samples	Lipase	Cholesterol esterase	α-glucosidase
	IC_{50} (μg/mL)		
Optimized extract	38.918 ± 1.067	46.143 ± 1.216	231.474 ± 17.978
PC	38.131 ± 0.720	39.099 ± 1.493	401.648 ± 13.010

PC: Positive control orlistat for lipase inhibition, simvastatin for cholesterol esterase inhibition, and acarbose for α-glucosidase inhibition

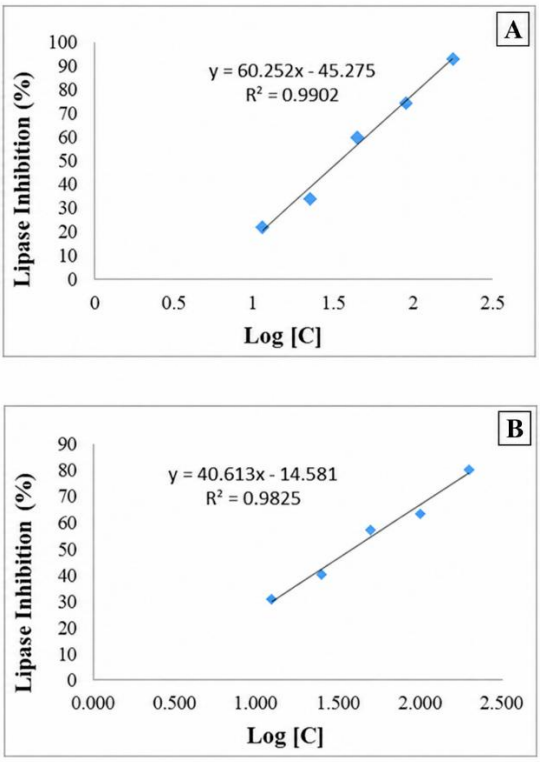

Figure 7. The calibration graphs of orlistat (A) and the optimized extract (B)

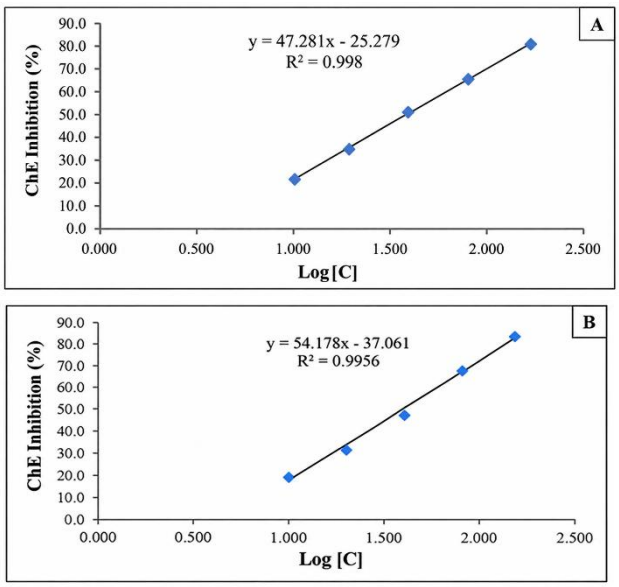

Figure 8. The calibration graphs of simvastatin (A) and the optimized extract (B)

The optimized Isabella grape pomace extract exhibited antioxidant activity as well as inhibitory effects against the three metabolic enzymes investigated. The CUPRAC and FRAP values of the optimized extract were 98.155 ± 0.262 and 71.279 ± 0.187 μmol TE/g dry extract, respectively, while its DPPH radical scavenging activity was determined with an SC_{50} value of 0.0569 ± 0.0006 mg/mL. In the enzyme inhibition assays, its pancreatic lipase inhibitory activity (IC_{50} = 38.918 ± 1.067 μg/mL) was comparable to that of orlistat (38.131 ± 0.720 μg/mL), while its cholesterol esterase inhibitory activity

(46.143 ± 1.216 µg/mL) was also close to that of simvastatin (39.099 ± 1.493 µg/mL). For α-glucosidase inhibition, the extract showed a lower IC_{50} value of 231.474 ± 17.978 µg/mL than acarbose (401.648 ± 13.010 µg/mL) under these experimental conditions. Overall, these findings suggest that the optimized extract possesses antioxidant capacity together with inhibitory activity toward enzymes involved in lipid and carbohydrate metabolism.

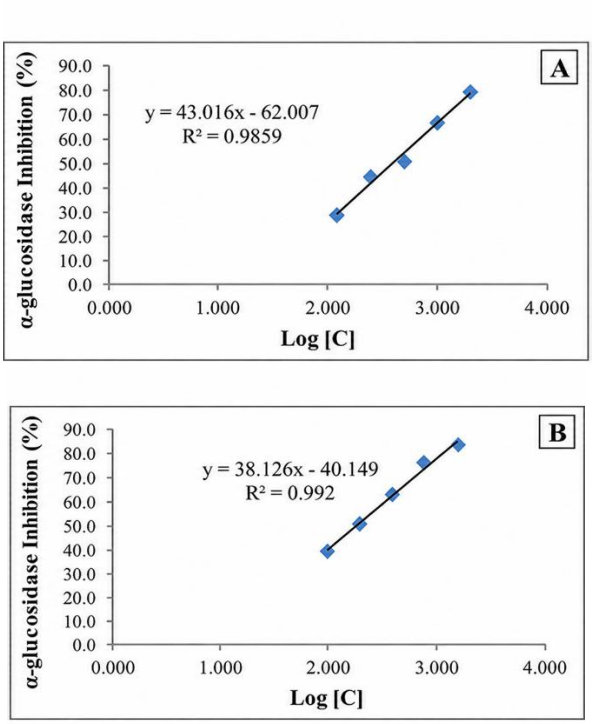

Figure 9. The calibration graphs of acarbose (A) and the optimized extract (B)

Vanillic acid was selected as the response variable for optimization primarily because it was consistently detected as a common and well-defined chromatographic peak in all extracts, allowing reliable monitoring of changes in extraction efficiency. This analytical rationale was further supported by the reported biological relevance of vanillic acid. Previous studies have demonstrated inhibitory effects of vanillic acid-containing polyphenol-rich extracts against carbohydrate- and lipid-hydrolyzing enzymes, including α-glucosidase, and pancreatic lipase [23–25,27–30]. Besides, several studies have supported a relationship between vanillic acid and α-glucosidase inhibition. Laaraj et al. (2022) [26] demonstrated α-glucosidase inhibitory activity of vanillic acid itself, while Kewuyemi et al. (2025) [31] reported a significant positive association between vanillic acid content and α-glucosidase inhibitory activity. Consistently, Tsagkaris et al. (2025) [32], using phytochemical profiling combined with enzyme inhibition assays across several extracts, identified vanillic acid as one of the compounds strongly correlated with α-glucosidase inhibition. Further studies have also reported that extracts containing vanillic acid exhibit simultaneous inhibitory activity against multiple metabolic enzymes, including α-glucosidase and pancreatic lipase [25,29]. Notably, Swaraz et al. (2021) [25] identified vanillic acid as a prominent phenolic acid in the active extracts and further demonstrated its potential interactions with the investigated enzymes through molecular docking analysis. Beyond *in vitro* enzyme inhibition, the metabolic relevance of vanillic acid is also supported by some *in vivo* studies. Kumar et al. (2014) [43] reported that oral administration of vanillic acid (50 mg/kg) ameliorated dyslipidemia in hypertensive rats, plasma and tissue lipid profiles, increasing lipoprotein lipase and lecithin cholesterol acyltransferase activities, and reducing hepatic and renal HMG-CoA reductase activity. In addition, Swaraz et al. (2021) [25] reported significant glucose lowering effects of vanillic acid containing extracts in a diabetic animal model. Although these in vivo findings do not establish a direct causal relationship between vanillic acid and the specific digestive enzyme inhibitory activities investigated here, they provide complementary evidence for the broader metabolic relevance of vanillic acid and vanillic acid-containing phytochemical preparations.

The optimized Isabella grape pomace extract exhibited notable antioxidant activity. This antioxidant activity may be associated with the phenolic constituents in the extract. Indeed, previous studies on different *Vitis* species and grape-processing by-products have reported associations between phenolic content and antioxidant capacity, with *V. labrusca* samples in particular representing an important source of phenolic compounds with antioxidant properties [44–49]. Nevertheless, considerable variation may exist among the phenolic contents and antioxidant capacities reported in the literature. Such differences may be attributed to factors including grape cultivar or genotype, geographical origin, the plant material or by-product fraction investigated, extraction procedure, and solvent system.

The recovery of phenolic compounds is strongly influenced by extraction conditions, as also demonstrated in previous studies. Ultrasound-assisted extraction has been reported to enhance phenolic recovery compared with conventional extraction methods [50], while different solvent combinations have resulted in substantial differences in the antioxidant capacity of extracts obtained from *V. labrusca* seeds [51]. Optimization of extraction conditions in Isabella grapes has likewise been shown to affect phenolic content and biological activity [52]. These observations are consistent with the present findings, in which ethanol concentration, microwave power, and temperature were identified as influential variables affecting extraction.

Thus, the application of an experimental design approach may be important not only for improving extraction efficiency but also for facilitating the effective recovery of phenolic compounds, potentially contributing to the biological activity of the extract.

In addition to its antioxidant activity, the optimized extract showed inhibitory activity against pancreatic lipase, cholesterol esterase, and α-glucosidase. Previous studies have also demonstrated the effects of polyphenol-rich grape and grape by-product extracts on enzymes involved in lipid and carbohydrate digestion. Grape seed extracts have been reported to inhibit pancreatic lipase and lipoprotein lipase, with these effects suggested to involve the combined actions of flavonoids and procyanidins [15,18,53,54]. Similarly, grape pomace extracts have shown α-glucosidase inhibitory activity and have been associated with reductions in postprandial blood glucose levels in experimental diabetic models [55]. These findings support a possible relationship between the phenolic composition of grape pomace and the pancreatic lipase and α-glucosidase inhibitory activities observed in the present study. However, given the phenolic contents of the extract, the observed activities should not be attributed to a single compound and may instead reflect the individual, additive, or synergistic contributions of multiple constituents.

Previous *in vivo* studies also provide supportive evidence regarding the metabolic and oxidative stress-related effects of grape-derived polyphenols. Grape pomace extracts have been reported to improve fasting and postprandial glucose levels, lipid profiles, and oxidative stress-related parameters in experimental models of diabetes [55,56]. Similarly, animal studies using *V. labrusca* leaves, grape juice, and grape-derived phenolic fractions have reported reduced oxidative stress, enhanced antioxidant defense, and improvements in certain lipid-related parameters [57–60]. These findings provide biological context for the antioxidant and enzyme inhibitory activities observed in the present study. However, as the current findings are based on *in vitro* assays, they should not be directly extrapolated to *in vivo* metabolic effects, and further studies are required to establish their physiological relevance.

4. Conclusions

In this study, a Design of Experiments approach was successfully applied to optimize the extraction of phenolic compounds from Isabella grape (*Vitis labrusca*) pomace from the Eastern Black Sea region of Türkiye. Ethanol concentration, microwave power, and extraction temperature were identified as the most influential extraction variables. The optimized extract contained several phenolic compounds, and its antioxidant, antilipidemic (pancreatic lipase and cholesterol esterase inhibition), and antidiabetic (α-glucosidase inhibition) activities were investigated. The extract exhibited antioxidant activity and showed inhibitory activity against pancreatic lipase, cholesterol esterase, and α-glucosidase, indicating that Isabella grape pomace represents a promising source of bioactive compounds with potential applications in the valorization of agro-industrial by-products. This study contributes to the existing literature by providing data on the antioxidant and metabolic enzyme inhibitory activities of Isabella grape pomace following experimental design-based extraction optimization, particularly regarding the less studied pancreatic lipase and cholesterol esterase inhibitory activities. However, the biological activities demonstrated in this study were based on *in vitro* assays, and the contribution of individual phenolic compounds to the observed effects was not experimentally established. Further studies involving bioactivity-guided characterization, mechanistic investigations, and *in vivo* models are therefore required to identify the compounds contributing to these activities and to establish their physiological relevance.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

Merve Badem: Writing-Original Draft, Conceptualization, Methodology, Validation, Investigation, Supervision.

Malek Al Faraj: Methodology, Validation, Investigation.

Seyda Kanbolat: Writing-Review & Editing, Formal analysis, Methodology, Validation, Investigation.

Nevin Ulaş Çolak: Methodology, Validation, Investigation.

Tuğba Subaş: Methodology, Validation, Investigation.

Ufuk Özgen: Writing-Review & Editing, Methodology.

Rezzan Aliyazıcıoğlu: Writing-Review & Editing, Methodology.

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Use of Generative AI

Generative artificial intelligence (AI) tools were used solely for language editing, grammar improvement, and enhancement of manuscript readability.

References

[1] P. Bridle, C.F. Timberlake, Anthocyanins as natural food colours—Selected aspects, Food Chem, 58, 1997, 103–109.

[2] G. Fan, Y. Han, Z. Gu, D. Chen, Optimizing conditions for anthocyanins extraction from purple sweet potato using response surface methodology (RSM), LWT-Food Sci Technol, 41, 2008, 155–160.

[3] M. Demirkol, Investigation of some physicochemical properties of yogurt supplemented with Isabella grape (*Vitis labrusca* L.) pomace during storage, Master's thesis, Ordu University, Institute of Science, Department of Food Engineering, Ordu, Türkiye, 2016.

[4] A. Güder, Determination of antioxidant activity of *Vitis labrusca*, Doctoral dissertation, Ondokuz Mayıs University, Institute of Science, Department of Chemistry, Samsun, Türkiye, 2012.

[5] S. Saltoglu, The use of bioactive extracts from Isabella grape by novel technologies in ayran production, Master's thesis, Ondokuz Mayıs University, Institute of Science, Department of Food Engineering, Samsun, Türkiye, 2014.

[6] A. Kurt, The effect of different ripening period on nutrient content in kokulu grape (*Vitis labrusca* L.) fruit, Master's thesis, Karadeniz Technical University, Institute of Science, Department of Biology, Trabzon, Türkiye, 2015.

[7] D. Sözbir, Production of probiotic oat milk enriched with Isabella grape (*Vitis labrusca* L.) juice and determination of survival of probiotic bacteria during storage period, Master's thesis, Ege University, Institute of Science, Department of Food Engineering, İzmir, Türkiye, 2021.

[8] D. Üneş, Investigation of phenolic composition and antioxidant effects of Isabella grape (*Vitis labrusca* L.) fruit, Master's thesis, Bartın University, Institute of Science, Department of Forest Industry Engineering, Bartın, Türkiye, 2016.

[9] K. Jayaprakasha, T. Selvi, K. Sakariah, Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts, Food Res Int, 36, 2003, 117–122.

[10] M. Pehluvan, M. Güleryüz, The importance of raspberries and blackberries for human health, Bahçe, 33, 2004, 51–57.

[11] B. De Souza, M. Thomazini, C. De Carvalho Balieiro, S. Fávaro-Trindade, Effect of spray drying on the physicochemical properties and color stability of the powdered pigment obtained from vinification byproducts of the Bordo grape (*Vitis labrusca*), Food Bioprod Process, 93, 2015, 39–50.

[12] M. Souza, L. Caldas, D. Tohidi, J. Molina, P. Souto, R. Fangueiro, A. Zille, Properties and controlled release of chitosan microencapsulated limonene oil, Rev Bras Farmacogn, 24, 2014, 691–698.

[13] S. Kuck, Z. Noreña, Microencapsulation of grape (*Vitis labrusca* var. Bordo) skin phenolic extract using gum Arabic, polydextrose, and partially hydrolyzed guar gum as encapsulating agents, Food Chem, 194, 2016, 569–576.

[14] S. Kuck, Z. Noreña, Application of gum arabic, β-cyclodextrin, and hydroxypropyl-β-cyclodextrin to microencapsulation by molecular inclusion of grape skin extract (*Vitis labrusca* var. Isabel), J Food Process Preserv, 43, 2019, e13874.

[15] B. Birari, K. Bhutani, Pancreatic lipase inhibitors from natural sources: Unexplored potential, Drug Discov Today, 12, 2007, 879–889.

[16] H. Wang, Y. Du, H. Song, α-Glucosidase and α-amylase inhibitory activities of guava leaves, Food Chem, 123, 2010, 6–13.

[17] M. Kazemipoor, M. Radzi, A. Cordell, I. Yaze, Safety, efficacy and metabolism of traditional medicinal plants in the management of obesity: A review, Int J Chem Eng Appl, 3, 2012, 288–292.

[18] P. Ercan, S. Nehir, Food ingredients in preventing obesity, Akad Gida, 12, 2014, 69–77.

[19] R.H. Myers, D.C. Montgomery, G. Geoffrey, M. Borror, M. Kowalski, Response surface methodology: A retrospective and literature survey, J Qual Technol, 36, 2004, 53–77.

[20] E. Pingo, Drug analysis and validation by high pressure liquid chromatography using experimental design, Master's thesis, Yıldız Technical University, Institute of Science, Department of Chemistry, Division of Analytical Chemistry, İstanbul, Türkiye, 2020.

[21] Ş. Polat, G. Gündemir, Ö. Karakaş, The resveratrol content of grape varieties under organic and conventional management, International Engineering and Natural Sciences Conference (IENSC 2019), Nov 6–8, Diyarbakır, Türkiye, 2019.

[22] N. Ulaş Çolak, M. Badem, S.Ö. Şener, Ş. Kanbolat, A. Yaşar, U. Özgen, A. Değirmenci, Optimization of phenolic acid extraction from *Origanum* species via novel deep eutectic solvents using response surface methodology, J Food Meas Charact, 2025, 1–12.

[23] M.S. Costamagna, I.C. Zampini, M.R. Alberto, S. Cuello, S. Torres, J. Pérez, M.A. Quispe, M.I. Isla, Polyphenols rich fraction from *Geoffroea decorticans* fruits flour affects key enzymes involved in metabolic syndrome, oxidative stress and inflammatory process, Food Chem, 190, 2016, 392–402.

[24] B. Prasad Pandey, S. Prakash Pradhan, K. Adhikari, LC-ESI-QTOF-MS for the profiling of the metabolites and in vitro enzymes inhibition activity of *Bryophyllum pinnatum* and *Oxalis corniculata* collected from Ramechhap District of Nepal, Chem Biodivers, 17, 2020, e2000155.

[25] A.M. Swaraz, F. Sultana, M.W. Bari, K.S. Ahmed, M. Hasan, M.M. Islam, M.A. Al-Bari, M.O. Raihan, Phytochemical profiling of *Blumea laciniata* (Roxb.) DC. and its phytopharmaceutical potential against diabetic, obesity, and Alzheimer's, Biomed Pharmacother, 141, 2021, 111859.

[26] N. Laaraj, M. Bouhrim, L. Kharchoufa, S. Tiji, H. Bendaha, M. Addi, S. Drouet, C. Hano, J.M. Lorenzo, M. Bnouham, M. Mimouni, Phytochemical analysis, α-glucosidase and α-amylase inhibitory activities and acute toxicity studies of extracts from pomegranate (*Punica granatum*) bark, a valuable agro-industrial by-product, Foods, 11, 2022, 1353.

[27] O.S. Ijarotimi, O.R. Fagoroye, T.D. Oluwajuyitan, Jute seed bioactive compounds: Amino acids, polyphenolics, antioxidants and hydrolyzing enzymes inhibitory property, J Future Foods, 3, 2023, 183–189.

[28] R. Danielski, F. Shahidi, Guava processing waste: Biological activity profile of a natural and sustainable source of phenolic antioxidants, Food Biosci, 56, 2023, 103294.

[29] R. Rani, D. Kaushik, F. Oz, G.D. Avila-Quezada, E.F. Abd_Allah, A. Hashem, M. Kumar, Polyphenol-rich extract from *Cyamopsis tetragonoloba* straw: Characterization, bioactivity, cytotoxicity, and anti-obesity potential, Environ Technol Innov, 2025, 104569.

[30] H. Liu, H. Zhang, M. Geng, D. Shi, D. Liu, Y. Jiao, Z. Lei, Y. Peng, The impact of cooking on antioxidant and enzyme activities in Ruichang yam polyphenols, Foods, 14, 2024, 14.

[31] Y.O. Kewuyemi, C.E. Chinma, H. Kesa, O.A. Adebo, Phenolic composition, functional groups, in vitro bioactivities, and techno-functional properties of whole and composite bioprocessed flours from cowpea, sorghum and orange-fleshed sweet potato, J Food Sci, 90, 2025, e70541.

[32] A.S. Tsagkaris, C. Cafarella, F. Rigano, A. Louckova, P. Dugo, L. Mondello, J. Hajslova, Bioactive compounds in 19 chili pepper varieties cultivated in Italy: Suspect screening and in vitro enzyme inhibitory effect, Food Res Int, 2025, 117051.

[33] N. Korkmaz, S.O. Sener, S. Akkaya, M. Badem, R. Aliyazicioglu, M. Abudayyak, U. Ozgen, Investigation of antioxidant, cytotoxic, tyrosinase inhibitory activities, and phenolic profiles of green, white, and black teas, Turk J Biochem, 44, 2019, 278–288.

[34] K. Slinkard, V.L. Singleton, Total phenol analysis: Automation and comparison with manual methods, Am J Enol Vitic, 28, 1977, 49–55.

[35] Z. Teng, X. Jiang, F. He, W. Bai, Qualitative and quantitative methods to evaluate anthocyanins, eFood, 1, 2020, 339–346.

[36] I.F. Benzie, J.J. Strain, Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids

and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration, Methods Enzymol, 299, 1999, 15–27.
[37] R. Apak, K. Güçlü, M. Özyürek, S.E. Karademir, E. Erçağ, The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas, Int J Food Sci Nutr, 57, 2006, 292–304.
[38] P. Molyneux, The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity, Songklanakarin J Sci Technol, 26, 2004, 211–219.
[39] Y. Bustanji, I.M. Al-Masri, M. Mohammad, M. Hudaib, K. Tawaha, H. Tarazi, H.S. AlKhatib, Pancreatic lipase inhibition activity of trilactone terpenes of *Ginkgo biloba*, J Enzyme Inhib Med Chem, 26, 2011, 453–459.
[40] M. Zor, S. Pekacar, D. Deliorman Orhan, Phytochemical content, antioxidant and enzyme inhibitory effects of *Nasturtium officinale*, J Fac Pharm Ankara Univ, 46, 2022, 114–128.
[41] U.D. Palanisamy, L.T. Ling, T. Manaharan, D. Appleton, Rapid isolation of geraniin from *Nephelium lappaceum* rind waste and its anti-hyperglycemic activity, Food Chem, 127, 2011, 21–27.
[42] J. Moutinho, I. Gouvinhas, R. Domínguez-Perles, A. Barros, Optimization of the extraction methodology of grape pomace polyphenols for food applications, Molecules, 28, 2023, 3885.
[43] S. Kumar, P. Prahalathan, M. Saravanakumar, B. Raja, Vanillic acid prevents the deregulation of lipid metabolism, endothelin 1 and up regulation of endothelial nitric oxide synthase in nitric oxide deficient hypertensive rats, Eur J Pharmacol, 743, 2014, 117–125.
[44] V.M. Burin, N.E. Ferreira-Lima, C.P. Panceri, M.T. Bordignon-Luiz, Bioactive compounds and antioxidant activity of *Vitis vinifera* and *Vitis labrusca* grapes: Evaluation of different extraction methods, Microchem J, 114, 2014, 155–163.
[45] N. Keskin, H. Bilir Ekbic, O. Kaya, S. Keskin, Antioxidant activity and biochemical compounds of *Vitis vinifera* L. (cv. 'Katıkara') and *Vitis labrusca* L. (cv. 'Isabella') grown in Black Sea Coast of Turkey, Erwerbs-Obstbau, 63, 2021, 115–122.
[46] M. Barriga-Sánchez, H.C. Hiparraguirre, M. Rosales-Hartshorn, Chemical composition and mineral content of Black Borgoña (*Vitis labrusca* L.) grapes, pomace and seeds, and effects of conventional and non-conventional extraction methods on their antioxidant properties, Food Sci Technol, 42, 2022, e120021.
[47] H. Tahmaz Karaman, D. Yuksel Kusku, G. Söylemezoğlu, H. Çelik, Phenolic compound and antioxidant capacity contents of *Vitis labrusca* L. genotypes, J Tekirdag Agric Fac, 19, 2022.
[48] A.M. Rocha, B.L. Viega, M. Fontana, L. Lucchetta, I.V. Almeida, V.E.P. Vicentini, E. Düsman, Grape bagasse extracts: Antioxidant and cytotoxic activity in HTC cells, Waste Biomass Valor, 14, 2023, 4071–4079.
[49] Y. Kara, M. Aybar, S. Kolaylı, Physicochemical, phenolic, and antioxidant profiles of *Vitis vinifera* L. and *Vitis labrusca* L. grapes from Artvin, Eastern Black Sea Region, J Food Sci, 90, 2025, e70435.
[50] T.K. Junior, C. De Moura, T.M. Cruz, M.B. Marques, M.A.V.d. Carmo, C.T.P. Deolindo, D. Granato, Optimization of the green chemistry-like extraction of phenolic compounds from grape (*Vitis labrusca* L.) and blackberry (*Rubus fruticosus* L.) seeds with concomitant biological and antioxidant activity assessments, Plants, 12, 2023, 2618.
[51] P.V. Dalposso, C.M.d. Aguiar, A.S. Torquato, T.S. Tiuman, C.A. Martin, R.F. Zara, S.M. Cottica, Optimization of antioxidant extraction and characterization of oil obtained by pressing cold from *Vitis labrusca* seeds, Food Sci Technol, 42, 2021, e47420.
[52] M.D. Vélez, M.A. Llano-Ramirez, C. Ramón, J. Rojas, C. Bedoya, S. Arango-Varela, M. Gil, Antioxidant capacity and cytotoxic effect of an optimized extract of Isabella grape (*Vitis labrusca*) on breast cancer cells, Heliyon, 9, 2023, e16540.
[53] A. Moreno, N. Ilic, A. Poulev, L. Brasaemle, K. Fried, I. Raskin, Inhibitory effects of grape seed extract on lipases, Nutrition, 19, 2003, 876–879.
[54] A. Tucci, J. Boyland, G. Halford, The role of lipid and carbohydrate digestive enzyme inhibitors in the management of obesity: A review of current and emerging therapeutic agents, Diabetes Metab Syndr Obes, 3, 2010, 125–143.
[55] S. Hogan, L. Zhang, J. Li, S. Sun, C. Canning, K. Zhou, Antioxidant rich grape pomace extract suppresses postprandial hyperglycemia in diabetic mice by specifically inhibiting alpha-glucosidase, Nutr Metab, 7, 2010, 71.
[56] M. Sabadashka, D. Chala, G. Chrzanowski, J. Cichoński, N. Sybirna, Beneficial effects of grape pomace extract on hyperglycemia, dyslipidemia, and oxidative stress in experimental diabetes mellitus, Molecules, 30, 2025, 4183.
[57] M.A. Hort, E.Z. Schuldt, Â.C. Bet, S. DalBó, J.M. Siqueira, C. Ianssen, M. Maraschin, Anti-atherogenic effects of a phenol-rich fraction from Brazilian red wine (*Vitis labrusca* L.) in hypercholesterolemic low-density lipoprotein receptor knockout mice, J Med Food, 15, 2012, 936–944.
[58] M.G. Cardozo, N. Medeiros, D. Dos Santos Lacerda, D.C. De Almeida, J.A.P. Henriques, C. Dani, C. Funchal, Effect of chronic treatment with conventional and organic purple grape juices (*Vitis labrusca*) on rats fed with high-fat diet, Cell Mol Neurobiol, 33, 2013, 1123–1133.
[59] D. Dos Santos Lacerda, C.F. Santos, A.S. Oliveira, R. Zimmermann, R. Schneider, F. Agostini, R. Gomez, Antioxidant and hepatoprotective effects of an organic grapevine leaf (*Vitis labrusca* L.) extract in diabetic rats, RSC Adv, 4, 2014, 52611–52619.
[60] L.K. Gonçalves, G. Bortolato, R.D. Braccini Neto, M. Rocha Frusciante, C. Funchal, C. Dani, Grape juice consumption with or without high fat diet during pregnancy reduced the weight gain and improved lipid profile and oxidative stress levels in liver and serum from Wistar rats, Beverages, 4, 2018, 78.