



Sustainable valorization of *Vitis labrusca* L. seeds: optimized phenolic extraction and utilization in antioxidant-enriched yogurt

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Abstract

This study aimed to optimize the extraction of phenolic compounds from *Vitis labrusca* L. seeds and to evaluate their functional effects in yogurt. A face-centered central composite design under Response Surface Methodology was applied using liquid-to-solid ratio and extraction temperature as variables. Total phenolic content (TPC) and DPPH IC₅₀ were used as response parameters. Optimal conditions yielded 969.30 mg GAE/g TPC and 13.71 mg/mL IC₅₀. The extract was incorporated into yogurt (0.5% and 1.0%, w/w), and physicochemical (pH), microbiological, antioxidant, and sensory properties were evaluated during 14 days of storage. Enriched samples showed significantly higher phenolic content and antioxidant activity compared to control ($p < 0.05$), while maintaining microbiological safety and acceptable sensory quality. The 0.5% formulation provided the best balance between functionality and sensory attributes. These findings demonstrate the potential of *V. labrusca* seed extract as a natural functional ingredient in dairy systems.

Keywords *Vitis labrusca* L. · Seed extract · Phenolic optimization · Yogurt fortification · Antioxidant activity

Introduction

Grape seeds are among the richest plant sources of dietary polyphenols, including proanthocyanidins (PACs), catechin/epicatechin, and gallic acid derivatives, which together confer potent antioxidant and antimicrobial activity. Grape seed extracts (GSE) typically exhibit higher total phenolic content and stronger radical-scavenging ability than skins or stems, while inhibiting foodborne bacteria through membrane disruption and metal-chelation mechanisms [18, 31, 34, 35]. These properties make grape seeds valuable raw materials for functional food fortification and natural preservation. Although most research focuses on *Vitis vinifera*, non-vinifera species such as *Vitis labrusca*—widely cultivated in humid regions—generate abundant processing by-products with distinctive phenolic profiles rich in anthocyanins and flavan-3-ols. However, only a limited number of studies have specifically focused on the optimization of phenolic extraction from *Vitis labrusca* seeds and their

application in food systems [11, 19]. Strategic valorization of these seeds could therefore broaden the range of clean-label antioxidants and antimicrobials for functional food development [11, 26].

Solid–liquid extraction (SLE) remains the most practical approach for polyphenol recovery at laboratory and pilot scales, despite advances in assisted methods such as ultrasound, microwave, pulsed-electric-field, and supercritical extraction. Among SLE variables, the solid-to-liquid (S/L) ratio governs mass-transfer efficiency, whereas temperature affects diffusion and solubility but can accelerate phenolic degradation if excessive. Studies confirm these as the most influential factors for grape seed phenolic yield ([6, 19]; Rajha et al., 2014). Response Surface Methodology (RSM) efficiently quantifies main, quadratic, and interaction effects and has proven effective in optimizing phenolic extraction from grape seeds and pomace through ANOVA-based model validation. However, few works have focused specifically on S/L ratio and temperature for *V. labrusca* seeds or validated optimized extracts in dairy matrices [3]; Gubsky et al., n.d.; Iglesias-Carres et al., [15]. Yogurt provides an ideal carrier: it is protein-rich, consumer-familiar, and offers cold-chain stability. Fortification with grape phenolics enhances TPC and antioxidant capacity, though protein–polyphenol interactions can influence color, astringency,

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Table 1 Factors of RSM design

Factor	Independent Variable	Levels		
		-1	0	+1
X	Solid:liquid ratio (w/v)	30:1	40:1	50:1
Y	Temperature (°C)	30	40	50

Table 2 Experimental design matrix provided by CCD

Run	Coded X	Coded Y	Solid:liquid ratio (w/v)	Temperature °C
1	-1	-1	30	30
2	+1	-1	50	30
3	-1	+1	30	50
4	+1	+1	50	50
5	-1	0	30	40
6	+1	0	50	40
7	0	-1	40	30
8	0	+1	40	50
9	0	0	40	40
10	0	0	40	40
11	0	0	40	40
12	0	0	40	40
13	0	0	40	40

and bioaccessibility, requiring combined evaluation of functionality and sensory quality during storage [16, 20, 38, 39, 44].

Despite the recognized potency of grape seed polyphenols, few studies have optimized *Vitis labrusca* L. seed extraction. The present work therefore aimed to optimize phenolic extraction using the solid-to-liquid ratio and temperature as variables under Response Surface Methodology (RSM), validate the predicted optima experimentally, and apply the optimized extract in yogurt. After yogurt production, samples were stored for 14 days and analyzed on days 1, 7, and 14 for microbiological counts, total phenolic content (TPC), DPPH radical-scavenging activity, and sensory quality. Addressing this integrated workflow bridges analytical optimization and application-ready functionality, ensuring ingredient performance and product acceptability in a real food matrix [14, 37]. Thus, RSM-optimized *V. labrusca* seed extract was validated and incorporated at 0.5% and 1.0% (w/w) into yogurt, whose refrigerated storage confirmed the extract's microbiological, antioxidant, and sensory stability—establishing a practical route for valorizing grape-seed phenolics in fermented dairy products [20, 38].

This study primarily focuses on phenolic extraction optimization and antioxidant functionality, while other quality parameters are considered beyond the present scope. However, detailed identification of individual phenolic compounds was not within the scope of this study.

Materials and methods

Materials

Vitis labrusca L. samples were obtained from the Arhavi district of Artvin during the 2024 harvest season and dried at room temperature for 10 days after harvest. The seeds of the dried samples were separated, washed with distilled water, and dried in a convection oven at 40 ± 1 °C until constant weight. The dried seeds were milled using a laboratory grinder (≤ 500 µm particle size) and stored in airtight, light-proof containers at 4 °C until extraction. DPPH (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich) was used for antioxidant activity analyses and Folin & Ciocalteu's phenol reagent (Sigma-Aldrich) was used for total phenol content analyses.

Methods

Experimental design and optimization

Phenolic extraction from *Vitis labrusca* L. seed powder was optimized using Response Surface Methodology (RSM) based on a Central Composite Design (CCD) to evaluate the combined effects of liquid-to-solid ratio (X, v/w) and extraction temperature (Y, °C) on total phenolic content (TPC) and antioxidant activity (DPPH IC₅₀). The design was face-centered with a total of 13 experimental runs, including five replicates at the design center to estimate pure error, with $\alpha = 1$ ensuring a rotatable and orthogonal design space. These replicates were used to estimate experimental error and improve the reliability of the fitted model. The factors and the levels are given in Table 1.

The factor levels were defined as follows: liquid-to-solid ratio, 30:1–50:1 (v/w); extraction temperature, 30–50 °C. The selected ranges for liquid-to-solid ratio and temperature were based on previous optimization studies on grape seeds and similar plant matrices [6, 32]b; [19]. All extractions were carried out using a hydroalcoholic solvent (methanol–water mixture, 70:30 v/v) on the plate shaker for 30 min at the designated temperature and ratio. Methanol was used solely as an extraction solvent at laboratory scale due to its high efficiency in phenolic recovery. The solvent was completely removed after extraction under reduced pressure, and the extract used for further application was solvent-free. After extraction, samples were centrifuged ($6500 \times g$, 15 min), and the supernatant was filtered (Whatman No. 42). The experimental design matrix is shown in Table 2. Each run was performed duplicate, and the mean values were used for statistical modeling. Although residual methanol was not analytically quantified, evaporation was performed

under reduced pressure at 40 °C until constant mass, minimizing the likelihood of solvent residues.

Model fitting and statistical analysis The experimental data were analyzed using Minitab v20.3 (Minitab LLC, State College, PA, USA). A second-order polynomial model was fitted to each response according to the equation:

$$\beta_{11}X^2 + \beta_{22}Y^2 + \beta_{12}XY$$

$$Y = \beta_0 + \beta_1X + \beta_2Y +$$

In the formula, Y represents the predicted response (TPC or DPPH IC₅₀), X and Y are the coded variables of solid-to-liquid ratio and extraction temperature, respectively, and β terms denote the regression coefficients for the intercept, linear, quadratic, and interaction effects. Model adequacy was evaluated by analysis of variance (ANOVA), lack-of-fit testing, and coefficients of determination (R^2 and adjusted R^2). The significance of each term was assessed using the F -test at the 95% confidence level ($p < 0.05$).

Validation of predicted optimum Predicted optimal conditions were experimentally validated in duplicate, which represents a limitation of the present study. However, the close agreement between predicted and experimental values and the low relative error indicate good model reliability. For the optimization stage, TPC and DPPH analyses were performed directly on the freshly obtained extracts prior to solvent removal. The rotary evaporation step was applied only to obtain a solvent-free extract for yogurt fortification and did not affect the optimization results. The observed TPC and DPPH IC₅₀ values were compared with predicted values, and relative error (%) was calculated as:

$$\text{Relative error (\%)} = \frac{|\text{Predicted} - \text{Observed}|}{\text{Predicted}} \times 100$$

The extract obtained under the simultaneous optimum was concentrated by rotary evaporation at 40 °C under reduced pressure to ensure complete removal of methanol. The resulting solvent-free extract was then used for yogurt enrichment. The solvent-free phenolic extract was stored at −20 °C until yogurt enrichment [3, 12, 15, 19, 24].

Yogurt production and enrichment

Yogurt was prepared from standardized cow's milk (3.0% fat, 3.2% protein). The milk was pasteurized at 90 °C for 10 min, cooled to 43 °C, and inoculated with a commercial

starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Chr. Hansen, Denmark). The solvent-free *V. labrusca* L. seed extract was added at 0.5% and 1.0% (w/w) concentrations, while a control without extract was produced under identical conditions. Only the extract obtained after complete removal of methanol was used in yogurt production. Fermentation proceeded at 43 °C until pH $\approx$ 4.6 [21], after which yogurts were cooled rapidly and stored at 4 °C for 14 days. All yogurts (control, 0.5%, 1.0%) were stored at 4 ± 1 °C. Analyses were performed on day 1, day 7, and day 14 of refrigerated storage to determine pH and antioxidant parameters, microbiological, antioxidant, and sensory parameters.

Analytical determinations

For analytical determinations, phenolic extracts obtained during optimization were used directly without solvent removal, whereas the solvent-free extract obtained after rotary evaporation was used only for yogurt enrichment.

pH determination The pH of yogurt samples was measured by directly immersing a calibrated pH electrode into the samples [28].

Microbiological enumeration Microbial quality of the yogurt samples was assessed using standard plate count techniques. Total mesophilic aerobic bacteria (TMAB), yeasts/molds, probable lactic acid bacteria, and coliforms, were enumerated following ISO methods (Iso 15214:1998, 2015; TS EN Iso 4833-2, 2014; TS Iso 4831, 2010; TS Iso 21527-1:2008, 2012). Microbiological analyses were performed on yogurt samples collected on days 1, 7, and 14 of storage. Samples were stored at 4 ± 1 °C throughout the experimental period and analyzed under identical laboratory conditions at each sampling time.

Phenolic extraction The extraction procedure for grape seed and yogurt phenolic compounds was adapted from the method described by Dai and Mumper [10] with certain modifications. Specifically, methanol–distilled water mixtures of varying concentrations were employed using a solid–liquid extraction technique. For the grape seeds, based on the design generated via RSM, 10 g of grape seed sample were weighed and homogenized using an Ultraturax homogenizer after the addition of 300, 400 and 500 mL of methanol–water mixtures containing 1% (v/v) 0.1 N HCl, with solvent concentration 70%. And for yogurt samples, 10 g of yogurt sample were mixed with 90 mL 70% methanol containing 1% HCl. The inclusion of HCl aimed to prevent oxidation prior to analysis. Extraction durations were adjusted 30 min. During extraction, all samples were

agitated at 150 rpm in an orbital shaker and subsequently centrifuged at 6500 rpm for 15 min. The resulting supernatants were filtered through Whatman No. 42 filter paper and stored at -20°C for subsequent determination of total phenolic content and antioxidant activity.

Antioxidant activity The DPPH radical scavenging activity of grape seed extracts was evaluated following the procedure described by Popović et al., [30], with slight modifications. Aliquots of 2.5, 5.0, and 7.5 $\mu\text{g/mL}$ were taken from the sample extracts, to which 500 μL of DPPH solution was added, and the total volume was adjusted to 2 mL with methanol. The mixtures were vortexed thoroughly to ensure uniformity and incubated in the dark at room temperature for 30 min. After incubation, the absorbance values were recorded at 517 nm using a spectrophotometer. The IC_{50} values were calculated from linear regression analysis of the inhibition percentage versus sample concentration. The regression models showed good linearity with coefficients of determination (R^2) higher than 0.99, indicating reliable estimation of antioxidant activity. The percentage of radical inhibition (%I) was calculated using the following equation:

$$\%I = \frac{(A_c - A)}{A_c} \times 100$$
 where A_c represents the absorbance of the control and A denotes the absorbance of the sample extract. The IC_{50} values, indicating the concentration required to scavenge 50% of DPPH radicals, were determined from the regression equation generated by plotting the percentage inhibition values against the corresponding sample concentrations.

The differences observed between total phenolic content and antioxidant activity may be attributed to the specific composition of phenolic compounds present in the extract. Since different phenolics exhibit varying antioxidant capacities, total phenolic content alone may not fully explain the radical scavenging activity. A detailed phenolic profiling using techniques such as HPLC-DAD or LC-MS would

provide further insight into the contribution of individual compounds. However, such analysis was beyond the scope of the present study. The discrepancy between TPC and DPPH activity can be attributed to differences in the redox potential and structural configuration of individual phenolic compounds (e.g., degree of hydroxylation, polymerization level).

Total phenolic content (TPC) Total phenolic content (TPC) was determined using the Folin–Ciocalteu method. A calibration curve was prepared using gallic acid standard solutions in the range of 0–200 mg/L. The calibration curve showed good linearity ($R^2 = 0.999$). Results were expressed as mg gallic acid equivalents (GAE)/g dry seed or/100 g yogurt. The calibration equation was $y = ax + b$, with $R^2 = 0.999$.

Sensory evaluation Sensory analyses of yogurt samples were conducted by a community-based panel of 11 adult panelists (trained by us in product and sensory analysis methods). A sensory panel form was prepared (Table 3), with some modifications, based on the criteria outlined by [36]. Using this form, yogurt samples were evaluated by the same panelists on days 1, 7, and 14 of storage for color and appearance, odor, water release, consistency, sourness, flavor, and overall acceptability.

Results and discussion

Model fitting and statistical evaluation

The experimental data for total phenolic content (TPC) and antioxidant activity (DPPH IC_{50}) were fitted to a second-order polynomial model, and the adequacy of each model was assessed by ANOVA and diagnostic statistics. The TPC model was highly significant ($F = 55.79$, $p < 0.001$) with a non-significant lack-of-fit ($p = 0.358$) and a high coefficient of determination ($R^2 = 0.976$), confirming an excellent correlation between predicted and observed values (Table 4). Similarly, the model for DPPH IC_{50} was statistically significant ($F = 41.83$, $p < 0.001$) with $R^2 = 0.968$ and a non-significant lack-of-fit ($p = 0.326$). These results verified the suitability of the quadratic model for describing the extraction behavior of *Vitis labrusca* L. seed phenolics within the studied experimental range.

The ANOVA for the quadratic model of TPC indicated that the model was highly significant ($p < 0.001$) with a high coefficient of determination ($R^2 = 0.9755$) and non-significant lack-of-fit ($p = 0.358$), confirming model adequacy. Both linear and quadratic effects were significant ($p < 0.05$), and the solid-to-liquid ratio exhibited the strongest influence

Table 3 Form of the sensory analysis

Full Name:

Date :

Please rate the numbered samples below on a scale of 1–9 in terms of appearance and color, flavor, consistency, sourness, separation of serum and overall acceptability.

9: Extremely good, 1: Extremely bad

Sensory Qualification	Sample No					
	---	---	---	---	---	---
Appearance and Color						
Flavor						
Consistency						
Sourness						
Separation of Serum						
General Acceptability						

Table 4 ANOVA results for TPC and DPPH

TPC					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	209497	41899	55.79	0.000
Linear	2	148093	74047	98.59	0.000
Solid: Liquid	1	119639	119639	159.29	0.000
Temperature °C	1	28454	28454	37.89	0.000
Square	2	40453	20226	26.93	0.001
Solid: Liquid*Solid: Liquid	1	38875	38875	51.76	0.000
Temperature °C*Temperature °C	1	1474	1474	1.96	0.204
2-Way Interaction	1	20951	20951	27.90	0.001
Solid: Liquid*Temperature °C	7	20951	20951	27.90	0.001
Lack-of-fit	3	5257	751		
Pure error	4	2722	907	1.43	0.358
Total	12	2536	634		
DPPH					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	160.283	32.057	41.83	0.000
Linear	2	114.956	57.478	75.00	0.000
Solid: Liquid	1	103.252	103.252	134.74	0.000
Temperature °C	1	11.704	11.704	15.27	0.006
Square	2	20.676	10.338	13.49	0.004
Solid: Liquid*Solid: Liquid	1	5.235	5.235	6.83	0.035
Temperature °C*Temperature °C	1	7.627	7.627	9.95	0.016
2-Way Interaction	1	24.651	24.651	32.17	0.001
Error	1	24.651	24.651	32.17	0.001
Lack-of-fit	7	5.364	0.766		
Pure error	3	2.910	0.970	1.58	0.326
Total	4	2.454	0.614		

on phenolic yield ($F=159.29$, $p<0.001$). The interaction between solid-to-liquid ratio and temperature was also significant ($p<0.01$), indicating synergistic behavior of the two factors. The ANOVA results for the DPPH IC₅₀ quadratic model demonstrated that the fitted model was highly significant ($p<0.001$) with a high coefficient of determination ($R^2 = 0.9676$) and a non-significant lack-of-fit ($p=0.326$), confirming the adequacy of the model. Both extraction parameters, solid-to-liquid ratio and temperature, significantly affected the antioxidant activity ($p<0.05$). Among them, the solid-to-liquid ratio was the most influential factor ($F=134.74$, $p<0.001$), followed by temperature ($F=15.27$, $p=0.006$).

The quadratic effects were also significant, suggesting a curvilinear relationship between extraction variables and DPPH response. Moreover, the significant interaction between solid-to-liquid ratio and temperature ($p<0.01$) indicated that the antioxidant activity was governed by the synergistic effects of both parameters rather than their

Table 5 Estimated regression coefficients for the coded variables (TPC and DPPH)

TPC					
Term	Coefficient	SE Coeff	T-Value	P-Value	VIF
Constant	591.3	11.4	51.96	0.000	
Solid:liquid ratio (X)	-141.2	11.2	-12.62	0.000	1.00
Temperature °C (Y)	-68.9	11.2	-6.16	0.000	1.00
X ²	118.6	16.5	7.19	0.000	1.17
Y ²	-23.1	16.5	-1.40	0.204	1.17
X×Y	72.4	13.7	5.28	0.001	1.00
DPPH IC ₅₀					
Term	Coefficient	SE Coeff	T-Value	P-Value	VIF
Constant	18.704	0.363	51.46	0.000	
Solid:liquid ratio (X)	4.148	0.357	11.61	0.000	1.00
Temperature °C (Y)	1.397	0.357	3.91	0.006	1.00
X ²	1.377	0.527	2.61	0.035	1.17
Y ²	1.662	0.527	3.15	0.016	1.17
X×Y	-2.482	0.438	-5.67	0.001	1.00

individual contributions. These findings confirm that the developed quadratic model reliably predicts the antioxidant activity of *Vitis labrusca* L. seed extracts within the studied experimental domain.

Regression analysis for TPC

The estimated regression coefficients for the coded variables (Table 5) showed that both the solid-to-liquid ratio and extraction temperature exerted highly significant effects on TPC ($p < 0.001$). The negative coefficients for the linear terms (-141.2 for solid: liquid ratio and -68.9 for temperature) indicate that an increase in either factor beyond the center level reduced phenolic yield, consistent with solute saturation and degradation of thermolabile compounds at higher solvent dilution or temperature [7, 33]. Conversely, the positive quadratic term for solid: liquid ratio (118.6 , $p < 0.01$) demonstrates a strong curvature effect, revealing an optimum region where solvent volume maximizes phenolic diffusion without excessive dilution.

The interaction term between solid: liquid ratio and temperature (72.4 , $p = 0.001$) was also significant, suggesting that the influence of temperature depends on solvent loading. This synergistic behavior is widely reported in grape seed extraction systems, where moderate heating accelerates diffusion and solvent penetration, provided that the solvent concentration is sufficient to prevent polymeric condensation [6, 13]. The resulting fitted equation for TPC in coded form is expressed as:

$$TPC = 591.3 - 141.2X - 68.9Y + 118.6X^2 - 23.1Y^2 + 72.4XY$$

The high t -values of the significant coefficients ($|t| = 6.16$ – 12.62 , $p < 0.001$) and low variance inflation factors ($VIF < 1.2$) confirm model stability and absence of multicollinearity.

Regression analysis for DPPH IC₅₀

The regression coefficients for antioxidant activity (Table 5) revealed that both solid-to-liquid ratio (4.148, $p < 0.001$) and temperature (1.397, $p = 0.006$) significantly influenced DPPH IC₅₀ values, with positive coefficients indicating that higher extraction intensity led to increased IC₅₀, i.e., lower antioxidant capacity. This inverse behavior relative to TPC suggests that excessive solvent or temperature may promote oxidative transformation of catechins and proanthocyanidins, thereby decreasing radical-scavenging efficiency [29].

Both quadratic terms were significant ($p < 0.05$), indicating curvature and confirming the presence of an optimum. The negative interaction term (-2.482 , $p = 0.001$) indicates that the combined effect of high temperature and large solvent ratio reduced IC₅₀ values, enhancing antioxidant activity through improved extraction of low-molecular-weight phenolics [23]. The final coded equation for the DPPH IC₅₀ response was:

$$IC_{50} = 18.70 + 4.15X + 1.40Y + 1.38X^2 + 1.66Y^2 - 2.48XY$$

The good agreement between the model predictions and experimental data, together with high t -statistics ($|t| > 3$) and $VIF < 1.2$, demonstrates that the model is both statistically valid and free from multicollinearity.

Comparative assessment and mechanistic implications

Both models indicate that solid-to-liquid ratio is the most influential extraction factor for *V. labrusca* seed phenolics, while temperature exerts a secondary but still significant effect. These findings align with previous optimization studies on grape seeds and other fruit by-products where solvent-to-solid ratio accounted for over 60:1 of the variability in phenolic yield [6]; [27]. The negative curvature observed in both responses confirms that phenolic recovery and antioxidant activity reach maxima within a moderate extraction domain—an outcome attributed to equilibrium mass transfer and avoidance of degradation [7, 13]. The simultaneous significance of linear, quadratic, and interaction terms, together with high R^2 and non-significant lack-of-fit, confirms the predictive reliability of the quadratic models.

Effects of extraction parameters on TPC

The influence of the solid-to-liquid ratio and extraction temperature on the total phenolic content (TPC) of *Vitis labrusca* L. seed extracts are illustrated in Fig. 1. A pronounced curvature was observed in the response surface, confirming the statistical significance of both the quadratic and interaction terms in the fitted model (Table 5). The solid-to-liquid ratio emerged as the dominant variable, showing a strong negative linear coefficient (-141.2 , $p < 0.001$) followed by a positive quadratic effect (118.6 , $p = 0.001$), indicating that phenolic yield first increased and then declined as the solvent proportion exceeded its optimum. This pattern suggests that at lower solvent volumes, the concentration gradient between solid and liquid phases limited mass transfer, whereas excessive solvent caused dilution and reduced solute–solvent interactions [29, 33].

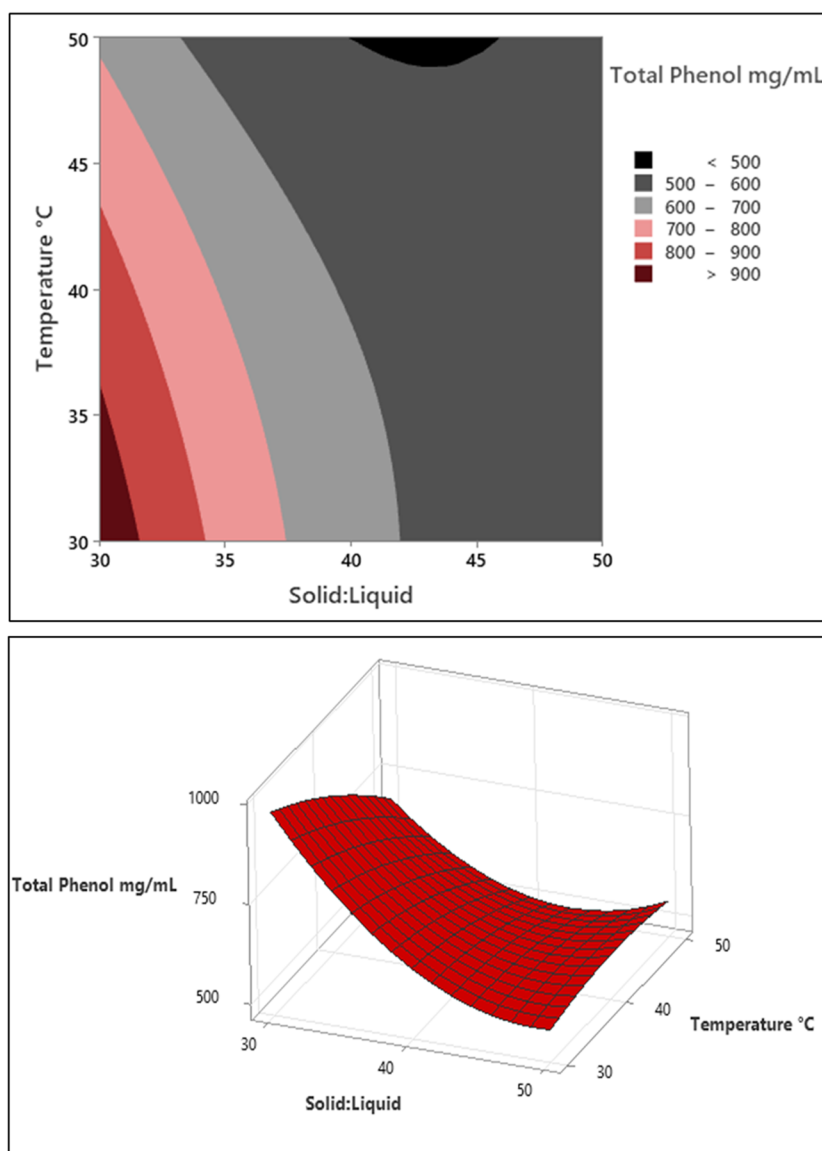
Extraction temperature also showed a significant effect (-68.9 , $p < 0.001$), although to a lesser extent than solvent loading. Moderate heating (around 40–45 °C) improved diffusivity and solvent penetration through seed cell walls, facilitating the release of bound phenolics such as catechin, epicatechin, and procyanidin oligomers [7]. However, higher temperatures beyond this range promoted degradation of thermolabile compounds and possible polymerization of flavan-3-ols, leading to a reduction in measurable TPC [13]. A significant interaction between solid-to-liquid ratio and temperature ($p = 0.001$) revealed that the positive impact of temperature was more pronounced at intermediate solvent levels. At excessive solvent ratios, the thermal effect diminished, probably because dilution lowered the effective concentration gradient and reduced contact between solvent molecules and phenolic-rich matrices. Similar synergistic behavior between temperature and solvent ratio has been reported for grape seeds [6] and other fruit by-products [27].

The model demonstrated that maximum TPC is achieved under moderate temperature and intermediate solid-to-liquid ratios, which balance efficient mass transfer with limited degradation. These findings are consistent with previously optimized extractions from *Vitis vinifera* and hybrid grape seeds, where optimal conditions generally occurred at 40–50 °C and solvent ratios between 35:1 and 45:1 (v/w) [1, 23]. The obtained behavior reinforces the suitability of *V. labrusca* seeds as a high-value phenolic source and validates the predictive accuracy of the RSM model for determining precise operating windows.

Effects of extraction parameters on DPPH IC₅₀

The response surface and contour plots in Fig. 2 describe the combined influence of the solid-to-liquid ratio and extraction temperature on the DPPH radical-scavenging activity

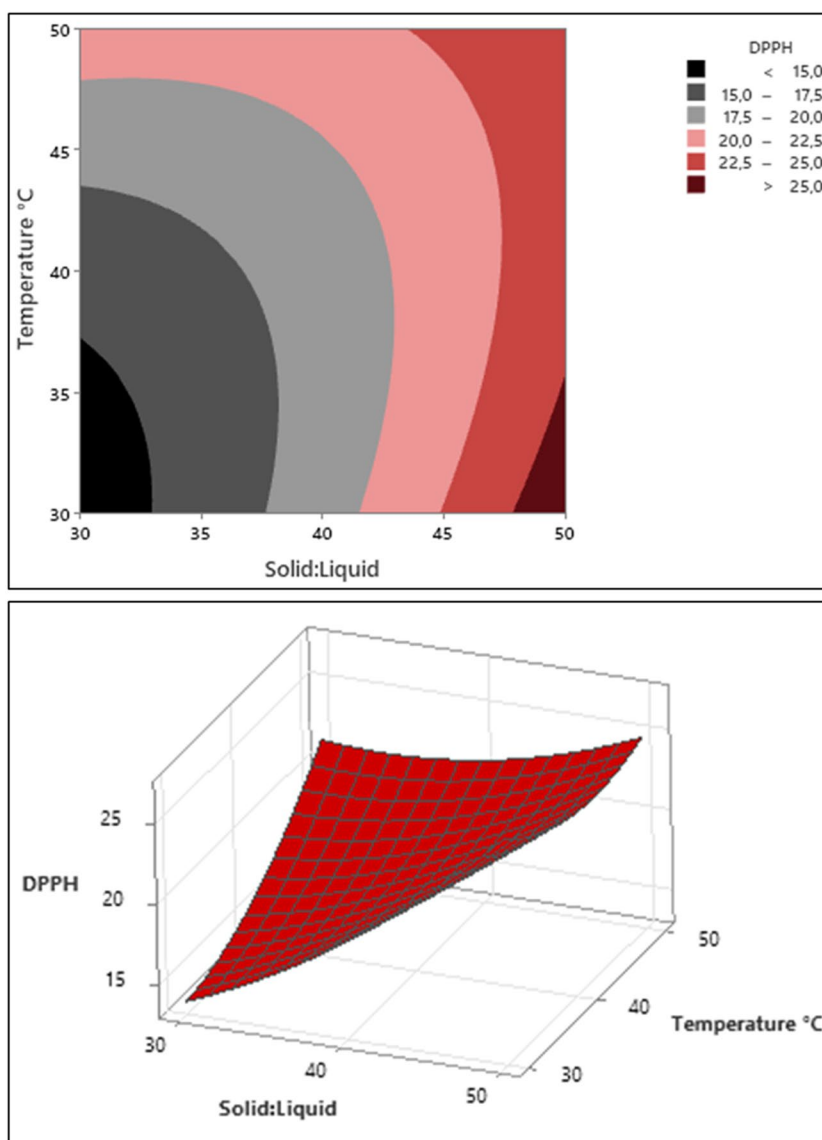
Fig. 1 TPC values surface and counter plots against temperature and solid:liquid ratio



(expressed as IC_{50}) of *Vitis labrusca* L. seed extracts. A lower IC_{50} value corresponds to higher antioxidant capacity; therefore, the descending regions on the plots indicate more efficient radical neutralization. Both extraction parameters exhibited statistically significant effects ($p < 0.05$; Table 4), and the overall curvature confirmed the presence of an optimal range rather than a monotonic trend. The solid-to-liquid ratio had the strongest influence, showing a positive linear coefficient (4.15, $p < 0.001$) and a significant quadratic component ($p = 0.032$), demonstrating that excessive solvent reduced radical-scavenging efficiency. At low solvent volumes, phenolic release was limited by insufficient solvent–matrix contact; however, when solvent volumes became excessive, the concentration of active antioxidants in solution decreased, resulting in weaker DPPH inhibition [22, 29].

Extraction temperature also significantly affected IC_{50} (1.40, $p = 0.006$). Moderate heating improved diffusion and solubility of low-molecular-weight phenolics such as gallic acid, catechin, and epicatechin, which exhibit high radical-scavenging efficiency [7, 13]. However, higher temperatures (above 45 °C) increased IC_{50} , suggesting thermal oxidation or condensation of monomeric flavan-3-ols into less active polymers [45]. The interaction term (solid-to-liquid $\times$ temperature) was highly significant (-2.48 , $p = 0.001$), revealing that temperature's effect depends on solvent loading. At intermediate solvent ratios (40:1–45:1), mild heating favored extraction of phenolics responsible for DPPH reduction, while excessive dilution or high temperature suppressed activity. This synergistic pattern matches reports for grape pomace and seed extracts, where moderate thermal

Fig. 2 DPPH values surface and counter plots against temperature and solid:liquid ratio



input coupled with limited solvent volume optimized antioxidant response [27, 32]/b.

The model demonstrates that antioxidant activity follows an inverse pattern to TPC, as over-extraction or aggressive heating can degrade phenolic radicals or alter their redox potential [1]. This supports that antioxidant capacity is governed not only by total phenolic concentration but also by phenolic composition and molecular structure. The response surface and contour (Fig. 2) both highlight a saddle-shaped domain where IC_{50} reaches its minimum, corresponding to a region of balanced extraction intensity. Such curvature is typical of phenolic-rich matrices where solvent polarity and temperature jointly govern antioxidant function rather than absolute phenolic concentration [9]. These findings align with the growing consensus that extraction efficiency and antioxidant capacity are not linearly coupled: the qualitative composition of the extract—particularly the proportion

of ortho-dihydroxylated and conjugated phenolics—plays a decisive role in free-radical neutralization [8]. The significant linear, quadratic, and interaction effects observed here thus confirm that *V. labrusca* seed phenolics respond predictably to multivariate optimization and that response-surface modeling is an appropriate approach for capturing their complex extraction dynamics.

Optimization and validation of the model

Numerical optimization using the desirability function in Minitab v20.3 was conducted to determine the extraction conditions that would maximize total phenolic content (TPC) and minimize DPPH IC_{50} . The individual optimization for each response yielded the same predicted optimal region, corresponding to a liquid-to-solid ratio of 30:1 (v/w) and an extraction temperature of 30 °C. Under these

Table 6 Validation of model predictions for optimal extraction of *Vitis labrusca* L. seed phenolics

Response	Optimization type	Optimum conditions (X, Y)	Predicted value	Observed value (mean $\pm$ SD)	Relative error (%)	Desirability
TPC (mg GAE/L)	Single-response	30:1 (w/v), 30 °C	969.30	958.23 $\pm$ 3.12	1.15	0.9992
DPPH IC ₅₀ (mg/mL)	Single-response	30:1 (w/v), 30 °C	13.71	13.58 $\pm$ 0.09	0.95	0.9926
TPC (mg GAE/L)	Simultaneous	30:1 (w/v), 30 °C	962.75	956.34 $\pm$ 2.84	0.67	0.9959
DPPH IC ₅₀ (mg/mL)	Simultaneous	30:1 (w/v), 30 °C	13.26	13.11 $\pm$ 0.10	1.13	0.9959

Table 7 Changes in total phenolic content (mg GAE/L) of yogurts during 14 days of storage

Day	Control (0 %)	0.5 % extract	1 % extract
1	52.26 $\pm$ 0.41 ^a	63.13 $\pm$ 0.36 ^a	71.39 $\pm$ 0.55 ^a
7	48.78 $\pm$ 0.28 ^b	57.91 $\pm$ 0.31 ^b	62.70 $\pm$ 0.44 ^b
14	47.91 $\pm$ 0.33 ^b	55.30 $\pm$ 0.29 ^b	60.52 $\pm$ 0.40 ^b

-Different superscript letters in the same row indicate significant differences ($p < 0.05$)

conditions, the model predicted a TPC of 969.30 mg GAE/g (desirability=0.9992) and a DPPH IC₅₀ of 13.71 mg/mL (desirability=0.9926). Validation experiments conducted in duplicate produced 958.23 mg GAE/g for TPC and 13.58 mg/mL for DPPH IC₅₀, resulting in relative errors of 1.15% and 0.95%, respectively. These low deviations confirm the high predictive capability of the quadratic models.

Simultaneous multi-response optimization, performed to obtain a balanced condition for both phenolic yield and antioxidant activity, also pointed to the same factor combination (solid-to-liquid=30:1; temperature=30 °C) with a composite desirability of 0.9959. The validation of model predictions for optimal extraction is given in Table 6.

At this joint optimum, the model predicted 962.75 mg GAE/g TPC and 13.26 mg/mL IC₅₀, while validation runs yielded 956.34 mg GAE/g and 13.11 mg/mL, with respective relative errors of 0.67% and 1.13%. Such close agreement between predicted and experimental results confirms that the established quadratic equations can reliably predict extraction efficiency within the studied domain. The excellent agreement between predicted and observed outcomes aligns with reports from comparable grape seed extraction studies, where RSM-derived models achieved prediction errors below 5% and desirability indices exceeding 0.95 [6, 7, 27]. The high composite desirability values (> 0.99) demonstrate that the experimental design achieved near-optimal performance, with operating conditions located well within the model's feasible region rather than at its boundaries—an indication of robust model behavior [4]. These validated conditions were subsequently used to produce the solvent-free phenolic extract incorporated into yogurt formulations.

Table 8 DPPH IC₅₀ (mg/mL) of yogurts during refrigerated storage

Day	Control (0 %)	0.5 % extract	1 % extract
1	10.26 $\pm$ 0.12 ^a	8.38 $\pm$ 0.09 ^{ab}	5.73 $\pm$ 0.06 ^{ab}
7	10.06 $\pm$ 0.10 ^a	9.49 $\pm$ 0.11 ^a	5.12 $\pm$ 0.08 ^b
14	8.94 $\pm$ 0.09 ^b	6.48 $\pm$ 0.07 ^b	6.23 $\pm$ 0.06 ^a

-Different superscript letters in the same row indicate significant differences ($p < 0.05$)

Application of optimized extract in yogurt

Total phenolic content (TPC)

The incorporation of the optimized *Vitis labrusca* L. seed extract significantly increased ($p < 0.05$) the TPC of yogurt samples throughout refrigerated storage (Table 7). On day 1, the TPC of control yogurt was 52.26 mg GAE/100 g, whereas yogurts enriched with 0.5% and 1% extract contained 63.13 and 71.39 mg GAE/100 g, respectively.

The higher phenolic retention in extract-fortified yogurts persisted through day 14, when control samples decreased to 47.91 mg GAE/100 g, compared to 55.30 and 60.52 mg GAE/100 g for the 0.5% and 1% treatments. A gradual decline in TPC during storage was observed for all groups, likely reflecting oxidative loss and binding of phenolics to milk proteins [40]. Nevertheless, extract-fortified samples retained approximately 10–20% higher phenolic level than controls, demonstrating the chemical stability of the grape seed polyphenols in the yogurt matrix.

Antioxidant activity (DPPH IC₅₀)

A strong dose-dependent enhancement in antioxidant activity was observed following extract addition (Table 8). On day 1, the DPPH IC₅₀ decreased from 10.26 mg/mL in the control to 8.38 mg/mL (0.5%) and 5.73 mg/mL (1%), confirming that lower IC₅₀ values correspond to higher radical-scavenging ability. Throughout 14 days, antioxidant activity remained significantly higher ($p < 0.05$) in the fortified yogurts, with the 1% formulation consistently showing the greatest efficiency.

A slight increase in IC₅₀ over time suggested limited degradation of extract antioxidants or interaction with casein micelles [5]. These results corroborate previous findings

where polyphenols effectively enhanced oxidative stability and bioactivity of fermented dairy systems. These findings clearly indicate that the incorporation of *Vitis labrusca* seed extract enhances the functional antioxidant capacity of yogurt. The observed reduction in IC₅₀ values confirms improved radical-scavenging efficiency, which can be attributed to the presence of phenolic compounds such as catechins and proanthocyanidins. This demonstrates that the extract acts as an effective natural antioxidant ingredient in the yogurt matrix, supporting its use in functional dairy product development.

Microbiological quality

Microbial counts confirmed that the addition of grape seed extract did not adversely affect the viability of starter cultures (Table 9). Lactic acid bacteria remained dominant throughout storage, increasing from 7.0 to 7.1 log CFU/g on day 1 to 8.3–8.5 log CFU/g on day 14 across all samples. This increase may be attributed to continued metabolic activity and residual post-fermentation of starter cultures during refrigerated storage. Such increases have been reported in early storage due to post-acidification and continued metabolic activity of starter cultures under refrigeration conditions.

Total mesophilic aerobic counts decreased marginally by the end of storage, while yeasts and molds declined from approximately 4.8 log CFU/g to around 3.0 log CFU/g.

No coliforms or *Enterococcus* species were detected, confirming hygienic quality.

Table 9 Microbiological profile (log CFU/g) of yogurts during refrigerated storage

Day	Sample	TMAB	Prob. <i>Lactobacillus</i> spp.	Prob. <i>Lactococcus</i> spp.	Total Yeast/mold
1	Control	4.59 ± 0.12 ^b	7.04 ± 0.09 ^a	4.39 ± 0.07 ^b	4.88 ± 0.11 ^a
	0.5 % extract	5.51 ± 0.10 ^a	7.14 ± 0.08 ^a	5.57 ± 0.05 ^a	4.91 ± 0.10 ^a
	1 % extract	4.04 ± 0.13 ^b	7.10 ± 0.06 ^a	4.49 ± 0.06 ^b	4.76 ± 0.09 ^a
7	Control	4.41 ± 0.11 ^b	7.84 ± 0.07 ^a	5.10 ± 0.08 ^a	4.91 ± 0.10 ^a
	0.5 % extract	4.85 ± 0.12 ^a	7.91 ± 0.09 ^a	4.87 ± 0.07 ^a	4.78 ± 0.08 ^{ab}
	1 % extract	4.46 ± 0.10 ^b	7.81 ± 0.08 ^a	4.85 ± 0.06 ^a	4.46 ± 0.07 ^b
14	Control	3.28 ± 0.09 ^{ab}	8.18 ± 0.10 ^a	2.98 ± 0.08 ^b	2.98 ± 0.08 ^a
	0.5 % extract	3.23 ± 0.08 ^b	8.52 ± 0.09 ^a	3.28 ± 0.09 ^b	3.25 ± 0.06 ^a
	1 % extract	3.02 ± 0.07 ^b	8.34 ± 0.11 ^a	3.02 ± 0.07 ^b	3.02 ± 0.07 ^a

-Different superscript letters within the same day indicate significant differences among treatments ($p < 0.05$)

Interestingly, the 1% extract group showed a small but statistically insignificant suppression of yeast/mold populations, potentially due to residual phenolic antimicrobial activity [20]. The absence of inhibitory effects on lactic acid bacteria suggests that the phenolic extract did not interfere with starter culture viability, which is essential for maintaining yogurt quality. Additionally, the slight reduction in yeast and mold counts may indicate a mild antimicrobial effect of phenolic compounds, contributing to product stability during storage.

Although a decline in lactic acid bacteria may be expected during prolonged storage, increases have also been reported in fermented dairy systems during early or intermediate storage due to continued viability and residual fermentation activity under refrigeration. Therefore, the increase observed in the present study was interpreted as continued starter culture activity rather than a negative microbiological deviation. Nevertheless, this point should be further confirmed in future studies with broader replication and extended storage monitoring.

Sensory evaluation

The sensory characteristics of yogurts fortified with *Vitis labrusca* L. seed phenolic extract are presented in Fig. 3. Evaluations were performed by a semi-trained panel ($n = 11$) using a nine-point hedonic scale (1 = “extremely bad” to 9 = “extremely good”) to assess appearance, color, aroma, flavor, texture, and overall acceptability. Results indicated that the inclusion of phenolic extract at both 0.5% and 1.0% (w/w) had no adverse effects ($p > 0.05$) on the major sensory attributes compared to the control yogurt, demonstrating good sensory compatibility of the grape seed polyphenols with the dairy matrix. In terms of appearance and color, a slight but perceptible difference was observed as extract concentration increased. The 1% formulation exhibited a faint beige–brown tone, attributed to the natural pigments and tannins present in the extract. However, panelists rated its visual appeal as natural and acceptable (mean = 7.20 ± 0.45), suggesting that color modification did not reduce product desirability. Similar outcomes have been reported by Amirdivani and Baba [2] and Bertolino et al., [5], who found that plant polyphenols imparted subtle, consumer-acceptable coloration to yogurts without decreasing overall preference.

Aroma and flavor scores revealed mild phenolic and fruity notes in extract-enriched yogurts, more noticeable at the 1% level. The 0.5% formulation, however, maintained a flavor profile most comparable to the control (mean = 7.65 ± 0.38) and was described as “pleasantly mild.” This concentration provided an optimal balance between functional enrichment and sensory neutrality. The higher extract level

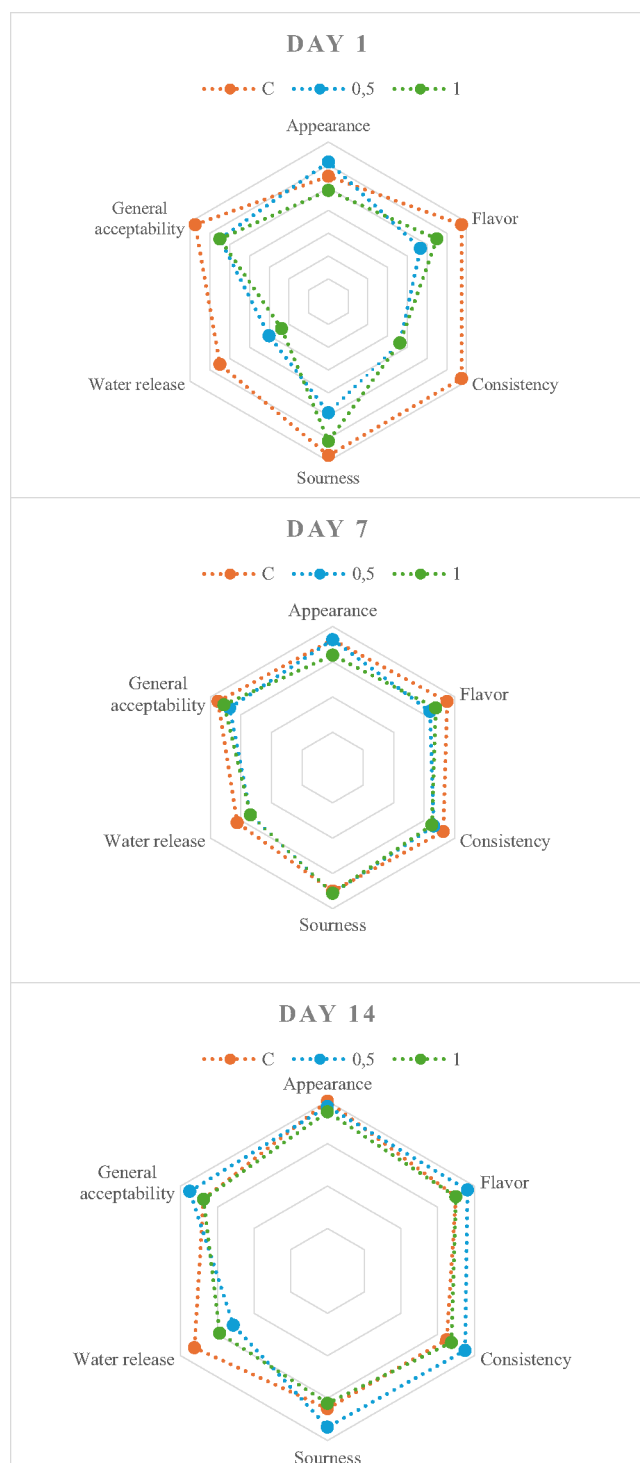


Fig. 3 Radar plot representing sensory scores (appearance, color, aroma, flavor, texture, and overall acceptability) of control and phenolic-enriched yogurts (0.5 % and 1 % *Vitis labrusca* L. seed extract)

(1%) introduced a slight astringency and bitterness—typical of condensed tannins—but remained within acceptable sensory thresholds (mean flavor score = 6.95 ± 0.42). Polyphenol–protein interactions are responsible for this perception,

as the binding of grape seed catechins and procyanidins to milk caseins can modulate taste intensity [5, 40].

For texture, all samples achieved similar scores (7.25–7.70), indicating that extract incorporation did not alter yogurt viscosity, mouthfeel, or syneresis. This suggests that phenolic compounds did not interfere with protein gelation or exopolysaccharide formation during fermentation. The consistent textural integrity aligns with prior studies where grape or tea polyphenols stabilized the microstructure of fermented dairy systems by forming weak hydrogen bonds with casein micelles [25]. Overall acceptability scores ranged from 6.75 ± 0.35 (1%) to 7.75 ± 0.32 (0.5%), both significantly higher than the control ($p < 0.05$). Panelists described the fortified yogurts as having a “natural, slightly fruity” flavor and a “rich creamy texture.” These results confirm that the inclusion of grape seed extract can enhance sensory appeal when incorporated at moderate levels, providing both functional and organoleptic advantages. The 0.5% enrichment level was therefore identified as the optimal formulation, achieving a favorable balance between health-promoting attributes and consumer perception.

Overall, the results confirm that *Vitis labrusca* seed extract provides functional benefits by enhancing antioxidant properties while maintaining microbial and sensory quality, thereby fulfilling the main objective of this study.

Conclusion

This study optimized the extraction of phenolic compounds from *Vitis labrusca* L. (Isabella grape) seeds and demonstrated the functional potential of the resulting extract in yogurt. Response Surface Methodology identified a solid-to-liquid ratio of 30:1 (v/w) and an extraction temperature of 30 °C as the optimal conditions for maximizing phenolic yield and antioxidant activity. The validated quadratic models exhibited excellent predictive accuracy, with relative errors below 1.2% and desirability indices exceeding 0.99, confirming their reliability for process optimization. The solvent-free extract, incorporated into yogurt at 0.5% and 1% (w/w), significantly enhanced total phenolic content and antioxidant capacity while maintaining microbiological safety and sensory acceptability throughout 14 days of refrigerated storage. Among the tested formulations, the 0.5% level provided the most favorable balance between functional enhancement and consumer acceptability, indicating that grape seed phenolics can be effectively stabilized within the dairy matrix without adversely affecting fermentation or textural properties. This integrated approach—from extraction optimization to application in a real food system—demonstrates a sustainable strategy for valorizing grape seed by-products as natural antioxidants in functional

foods. Furthermore, these findings contribute to a broader understanding of how extraction parameters influence phenolic extract quality and their functional performance in fermented dairy systems. Future research should focus on bioavailability assessment, extended storage stability, and application in different dairy matrices to support the scalable use of *V. labrusca* phenolics in health-oriented food formulations.

Following these findings, several limitations should be acknowledged. Methanol was used as an extraction solvent at the laboratory scale due to its high efficiency; however, it was completely removed prior to food application. Future studies should prioritize the use of food-grade solvents such as ethanol and include residual solvent analysis (e.g., GC–MS) to confirm complete removal and ensure compliance with food safety standards. In addition, validation experiments were conducted in duplicate, which may limit statistical robustness; therefore, future work should include triplicate validation and report variability parameters such as standard deviation or coefficient of variation to improve reliability. Furthermore, the absence of detailed phenolic profiling represents another limitation of the present study; accordingly, future research should incorporate advanced analytical techniques (e.g., HPLC–DAD or LC–MS) to identify individual phenolic compounds and better elucidate the relationship between phenolic composition and antioxidant activity. Finally, additional quality parameters, including proximate composition, color, and viscosity, should be evaluated to provide a more comprehensive assessment of the developed product.

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Data availability No data was used for the research described in the article.

Declarations

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Consent for publication The author consent to publish this work.

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