Frontiers in Sustainable Food Systems

TYPE Original Research
PUBLISHED 10 June 2025
DOI 10.3389/fsufs.2025.1606602

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OPEN ACCESS

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RECEIVED 05 April 2025
ACCEPTED 26 May 2025
PUBLISHED 10 June 2025

CITATION
Yıkmış S, Türkol M, Tokatlı Demirok N, Tokatlı N, Rüzgar E, Mohamed Ahmed IA and Aljobair MO (2025) Sustainable valorization of yellow cherry juice using natural propolis and non-thermal techniques. *Front. Sustain. Food Syst.* 9:1606602. doi: 10.3389/fsufs.2025.1606602

Sustainable valorization of yellow cherry juice using natural propolis and non-thermal techniques

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This study investigated the effects of propolis enrichment and thermosonication conditions on bioactive components, amino acid profile, antioxidant capacity and sensory properties of yellow cherry juice. Temperature (40–50°C), time (4–10 min), amplitude (40–80%) and propolis concentration (40–80 mg/100 mL) were optimized as independent variables using response surface methodology (RSM). Principal component analysis (PCA) analysis revealed that thermosonicated samples (TS-YCJ) were positively correlated with functional components such as chlorogenic acid, caffeic acid, epicatechin and total soluble solids (TSS). The malic acid content reached its highest level at 1,174.38 mg/L in thermosonicated optimized propolis yellow cherry juice (TS-YCJ), whereas this value remained at 1,078.34 mg/L in the pasteurized samples. Thermosonication application significantly increased the antioxidant capacity measured by total phenolic content (TPC), total flavonoid content (TFC) and DPPH radical inhibition. While TPC content reached 268.72 mg GAE/L in thermosonicated optimized propolis yellow cherry juice samples, it remained at 256.27 mg GAE/L in control samples. Among the phenolic compounds, chlorogenic acid (35.42 mg/L) and caffeic acid (12.67 mg/L) increased significantly after thermosonication. In terms of amino acid profile, components such as proline (42.21 mg/L), glycine (38.45 mg/L) and phenylalanine (24.32 mg/L) were found at higher levels in control samples. In sensory analysis, thermosonication samples received high scores in terms of taste, odor and overall acceptability. High R^2 values (98.94–99.80%) reveal the strong explanatory power and reliability of the model. These findings indicate that thermosonication and propolis offer an effective combination to improve the functional properties, sensory quality and phenolic compound profile of yellow cherry juice.

KEYWORDS

yellow cherry juice, thermosonication, propolis, phenolic compounds, amino acid profile

1 Introduction

The sweet cherry (*Prunus avium* L.), a commercially valuable member of the Rosaceae family, is among the most widely appreciated and cultivated fruit species worldwide. Cherries are rich in bioactive compounds and essential minerals like phosphorus, calcium, magnesium, and potassium (Dirlewanger et al., 2012; Hayaloglu and Demir, 2015; Çağlar and Demirci, 2017; Gonçalves et al., 2017; Vignati et al., 2022).

Consumers have highly valued sweet cherries for their quality characteristics, such as sweetness, acidity, taste, firmness, and color, and their consumption has been associated with beneficial effects (Clodoveo et al., 2023). Thermal and non-thermal methods of preserving food, including cooking, sterilizing, pasteurizing, and aseptic packaging, are widely used to inactivate microorganisms (Rathnayake et al., 2025). Ultrasound has been proven to be a reliable, effective, and environmentally friendly technique (Guo et al., 2025). It causes thermal, chemical, mechanical, and cavitation effects through the transfer of ultrasound media. The thermal mechanism of ultrasound is such that, upon propagation through a medium, the vibration energy of the ultrasound is absorbed by the medium and converted into heat energy. This increase in the medium's temperature is due to the conversion of vibration energy to heat energy (Zhou et al., 2024). Fruit juices constitute primary sources of bioactive compounds such as polyphenols, antioxidants, carotenoids, and vitamins (Panigrahi et al., 2025).

In many studies, the possibilities of ultrasound on bioactive systems in fruit juices have been investigated and it has been observed that ultrasound treatment enriches bioactive compounds (Bhutkar et al., 2024; Ramírez-Corona et al., 2024; Yıkmış et al., 2024; Zhang et al., 2024). To date, there are no studies investigating the effects of ultrasound on propolis-enriched yellow cherry juice. Previous literature indicates that the combined application of moderate heat and ultrasound results in more favorable outcomes compared to the individual use of either treatment. In light of this, the objective of the present study was to optimize the thermosonication process for the production of high-quality propolis-enriched yellow cherry juice through Response Surface Methodology (RSM). Furthermore, a comprehensive analysis was conducted to compare several key parameters, including soluble solids (°Brix), pH, titratable acidity, organic acid and sugar profiles (HPLC), phenolic compound content (HPLC), free amino acid composition (LC–MS–MS), polyphenol oxidase (PPO) activity, and sensory properties. These parameters were assessed in yellow cherry juices subjected to thermal pasteurization, untreated, thermosonicated, and thermosonicated with propolis treatments. This study aims to investigate the potential of ultrasound-assisted processes in enhancing the quality and bioactive properties of propolis-enriched yellow cherry juice.

2 Materials and methods

2.1 Vegetable material

Stark Gold yellow cherries (*Prunus avium* L.) were harvested at full ripeness from an orchard located in Tekirdağ, Türkiye (40.6152° North latitude and 27.1136° East longitude). Fruits were selected based on uniform size and color, and free from physical damage or disease. After harvesting, the cherries were immediately transported to the laboratory under refrigerated conditions (4 ± 1°C), washed with distilled water to remove any surface contaminants, and used for processing within 24 h of collection. Analyses were conducted in the laboratories of Tekirdağ Namık Kemal University's Çorlu Vocational School, Tekirdağ, Türkiye. The organic propolis extract (33%) was purchased commercially without any special processing and stored at-20°C until the end of the study.

2.2 Preparation of yellow cherry juice concentrate and propolis addition

Fresh yellow cherry fruits were first crushed in a blender (ISO LAB Blender, 602.21.001) at low speed for 30 s, and the fruit pulp was obtained. The obtained fruit pulp was diluted with sterile water (1:1 ratio). The mixture was filtered. The prepared concentrates were stored in 50-mL sterile sample containers with lids at −18°C. Zero point 5 g of propolis samples were weighed on a precision balance, and each was transferred to a 50 mL glass bottle separately. Their final volumes were then completed to 20 mL with pure water. After the mixtures were thoroughly vortexed, they were incubated for 24 h by continuously shaking at 150 rpm at 60°C. After incubation, each mixture was centrifuged at 3,000 *g* for 10 min. The supernatants were then filtered through filter paper and passed through 0.45 µm membrane filters. The propolis extracts prepared in this manner were stored in the dark at 4°C in the refrigerator for use in necessary experiments and analyses. Propolis extract was first prepared and subsequently added to yellow cherry juice to obtain enriched samples. The ultrasound treatment was then applied to these enriched juice formulations. This sample was named as thermosonicated propolis yellow cherry juice. Yellow cherry juice samples were filled into 100 mL glass bottles, and the pasteurization process was carried out at 85 ± 1°C for 2 min using the Wisd brand WUC-D06H model water system from Daihan Company (Wonju, Korea). This sample was named as thermal pasteurized yellow cherry juice (P-YCJ) (Yıkmış et al., 2025). After thermosonication and pasteurization, the samples were quickly cooled in an ice bath to maintain the stability of the fruit juice samples and stored at −18 ± 1°C until analysis.

2.3 Experimental design of response surface methodology

Yellow cherry juice was processed using the response surface method Minitab Statistical Analysis Software (Minitab 18.1.1) to understand the effects of thermosonication and propolis extract addition on quality parameters. Response Surface Method (RSM) was used. Box–Behnken Design (BB) was selected as the experimental design, and a three-level, four-factor experimental design was created. This design is an independent quadratic design that does not include full or partial factorial designs. It is the design that requires the least amount of manipulation (Table 1). Model adequacy was evaluated based on the R^2 and corrected R^2 coefficients, lack-of-fit tests, and ANOVA results. The independent variables were determined as temperature (X_1), time (X_2), amplitude (X_3), and propolis extract (X_4).

TABLE 1 Independent variable values.

Independent variable	Level		
	−1	0	1
X_1: Temperature (°C)	40	45	50
X_2: Time (min)	4	7	10
X_3: Amplitude (%)	40	60	80
X_4: Propolis extract (mg/100 mL)	40	60	80

The temperature range (40–50°C) and time range (4–10 min) used in this study were chosen based on preliminary experiments and the thermal sensitivity of components in yellow cherry juice, with the aim of optimizing the efficiency of thermosonication while preserving heat-labile phenolic compounds and amino acids. An ultrasonicator device was used. Dependent variables were selected as pH, titratable acidity, °Brix, phenolic substances, flavonoid substances, antioxidant activity, PPO activity, organic acid and sugar components, and phenolic compound activity. The second-degree degree-polynomial equation shown in the equation below was used to create the model equations:

$$y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii} X_i^2 + \sum_{\substack{i=1 \\ i<j}}^{3}\sum_{j=1}^{3}\beta_{ij} X_i X_j$$

In the equation, Y is the dependent variable, βo is the intercept term, βi is the first-degree (linear) equation coefficient, βii is the second-degree equation coefficient, βij is the two-factor cross-interaction coefficient, and Xi and Xj are the independent variables.

2.4 Determination of bioactive compounds

TPC was determined through spectrophotometric analysis. The Folin–Ciocalteu method was utilized with modification. The measurement of the degree of absorption was conducted using a UV–VIS spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Victoria, Australia) at a wavelength of 765 nm. The calibration curve for TPC analysis, constructed using gallic acid as the standard, demonstrated a strong linear relationship with a correlation coefficient (R^2) exceeding 0.99. Subsequently, a transformation of the findings was performed, resulting in the subsequent formulation: milligrams of gallic acid equivalents per liter (mg GAE/L) (Singleton and Rossi, 1965). The quantification of total flavonoid content was performed using the aluminum chloride colorimetric analysis method. The calibration curve for TFC analysis, established using quercetin as the standard, exhibited a strong linear relationship with a correlation coefficient (R^2) exceeding 0.99. The quantity of flavonoids is expressed in milligrams of quercetin equivalents (CE) per liter (Zhishen et al., 1999). The determination of antioxidant capacity in Uruset apple juice was accomplished by employing the DPPH scavenging activity method as delineated by Singh et al. (2002) (Singh et al., 2002). The DPPH inhibition was reported in terms of percentage. It is the control sample of the untreated group.

2.5 Analysis of polyphenol oxidase enzyme (PPO) activity

A quantity of 0.1 mL of juice sample and 2.8 mL of a Na-phosphate buffer solution with a pH of 6.8 were added to the cuvette in the spectrophotometer's cuvette holder. The solution was left for a period of 3 min to facilitate the extraction of PPO. After the termination of the incubation period, 0.1 mL (0.1 M) of catechol solution was added and thoroughly mixed. Absorbance measurement was immediately initiated at 410 nm. Absorbance measurements were recorded at 15 s intervals, and a linear absorbance time graph was drawn using the data obtained for 5 min. The calculation of PPO enzyme activity in % was conducted by utilizing the slope of the graph, as outlined in the study by Cemeroğlu (2010).

2.6 Physicochemical analysis

The temperature of the samples was calibrated to a standard of 20°C, and the pH analysis was subsequently conducted using the Hanna Instruments HI 2002 pH/ORP device (AOAC, 1990). The determination of the amount of total soluble solids was achieved through the implementation of the refractometric method. The measurements were conducted at a temperature of 20°C, utilizing a PCE-032 brand refractometer. The results were subsequently expressed as °Brix (AOAC, 1990). The titratable acidity of the samples was determined by potentiometric titration, whereby 0.1 N NaOH solutions were titrated to a pH of 8.1 (Cemeroğlu, 2010). Analyses were performed in three parallels.

2.7 Amino acid content

The amino acid content was determined by the method described by Bilgin et al. (2019) with minor modifications (Bilgin et al., 2019). Amino acid analysis was performed using a liquid chromatography (LC) system (Agilent Technologies, Waldbronn, Germany). MS/MS analyses were performed using an Agilent 6460 triple quadrupole LC–MS system equipped with an electrospray ionization interface. A JASEM quantitative amino acid kit protocol (Sem Laboratory Devices Inc., Istanbul, Türkiye) was used to determine the composition of amino acid. The samples were analyzed using the device after filtration, without applying acidic hydrolysis or dilution. The results were expressed as milligrams per 100 g.

2.8 Analysis of organic acid and free sugars

The determination of the quantity of organic acids present in the samples was conducted following the methodology outlined by Castellari et al. (2000). This method was initiated with the establishment of standards within the anticipated concentration ranges for organic acids (i.e., citric, malic, and ascorbic acids) and free sugars (e.g., sucrose, glucose, and fructose). Citric and malic acids were determined at 210 nm, while ascorbic acid was measured at 243 nm, employing the DAD detector. The results are expressed in grams per 100 mL for organic acids. The quantity of sucrose in the samples was ascertained by the method delineated by Sturm et al. (2003). The analysis of sugars was conducted using the RID detector. The results obtained for sugar are expressed in grams per liter.

2.9 Analysis of phenolic compounds

The analysis of phenolic compounds was conducted utilizing an Agilent 1260 Infinity chromatograph equipped with a diode array detector (DAD). As stated in the study by Portu et al. (2017), the chromatography process was carried out using a C-18 Agilent column

(250 × 4.6 mm; 5 μm packing) (Portu et al., 2017). The column temperature was set to 30°C, and the flow rate was established at 0.80 mL/min. The levels of these compounds are given in μg/mL. The results about phenolic compounds are expressed as the mean of three sample analyses. The phenolic compounds analyzed in the study consisted of chlorogenic acid, phloridzin, gallic acid, caffeic acid, cinnamic acid, p-coumaric acid, quercetin, catechin, epicatechin, and ferulic acid. The calibration curves were developed for each phenolic compound in the concentration range from 2.5 to 250 mg/L. The calibration curves obtained exhibited a high level of linear relationship, as evidenced by correlation coefficients (R^2) that exceeded 0.99 for all compounds. The findings were expressed in micrograms per milliliter (μg/mL).

2.10 Sensory analysis

The thermosonicated yellow cherry juice, evaluated by the surface response method, was compared with thermal pasteurized yellow cherry juice and fresh yellow cherry juice. The study was conducted in two replicates. Panelists were asked to evaluate the taste, odor, color, texture, and general acceptability of the samples. Fifty panelists who evaluated the juice sample were studied (Petrou et al., 2012).

2.11 Statistical analysis

Statistical analyses of the study's comparative data were performed using SPSS 20.0 (SPSS Inc., Chicago, U.S.A.). Samples were compared using a One-way ANOVA multiple comparison Tukey test. The statistical significance level was determined as $p < 0.05$. The response surface method was analyzed using Minitab Statistical Analysis Software (Minitab 18.1.1).

3 Results and discussion

3.1 Optimization of bioactive compounds and PPO

This study evaluated the effects of temperature (X_1), time (X_2), amplitude (X_3), and propolis extract (X_4) on the bioactive properties of yellow cherry juice using Response Surface Methodology (RSM). The optimization process was carried out to increase Polyphenol oxidase (%) (PPO), total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (DPPH). Table 2 presents the measured responses used in the experimental design for RSM. The effects of the independent variables temperature (X_1), time (X_2), amplitude (X_3), and propolis (X_4) on the PPO (Equation 1), TPC (Equation 2), TFC (Equation 3) and DPPH (Equation 4) properties of yellow cherry juice are shown in the following equations.

$$\begin{aligned} PPO(\%) &= -58{,}94 + 4{,}112\,X_1 - 0{,}639\,X_2 + 0{,}6430\,X_3 \\ &+ 0{,}1599\,X_4 - 0{,}05093\,X_1X_1 - 0{,}13454\,X_2X_2 \\ &- 0{,}002836\,X_3X_3 - 0{,}004211\,X_4X_4 + 0{,}04450\,X_1X_2 \\ &- 0{,}005525\,X_1X_3 + 0{,}008750\,X_1X_4 + 0{,}003375\,X_2X_3 \\ &+ 0{,}005167\,X_2X_4 - 0{,}001206\,X_3X_4 \end{aligned} \tag{1}$$

$$\begin{aligned} TPC(\text{mg GAE/L}) &= 182.8 + 2.734\,X_1 - 9.83\,X_2 + 1.762\,X_3 \\ &- 0.125\,X_4 - 0.04888\,X_1X_1 - 0.1320\,X_2X_2 - 0.007652\,X_3X_3 \\ &- 0.007180\,X_4X_4 + 0.2403\,X_1X_2 - 0.02302\,X_1X_3 \\ &+ 0.02830\,X_1X_4 + 0.02225\,X_2X_3 \\ &- 0.01858\,X_2X_4 - 0.000669\,X_3X_4 \end{aligned} \tag{2}$$

$$\begin{aligned} TFC(\text{mg CE/L}) &= -25.14 + 3.053\,X_1 - 3.333\,X_2 \\ &+ 0.4504\,X_3 - 0.4479\,X_4 - 0.05145\,X_1X_1 \\ &- 0.08264\,X_2X_2 - 0.003662\,X_3X_3 - 0.002550\,X_4X_4 \\ &+ 0.06317\,X_1X_2 + 0.001850\,X_1X_3 + 0.018300\,X_1X_4 \\ &+ 0.00704\,X_2X_3 + 0.02062\,X_2X_4 - 0.002556\,X_3X_4 \end{aligned} \tag{3}$$

$$\begin{aligned} DPPH(\text{inhibition\%}) &= -113.21 + 6.222\,X_1 + 0.030\,X_2 \\ &+ 0.8489\,X_3 + 0.3180\,X_4 - 0.07492\,X_1X_1 \\ &- 0.20074\,X_2X_2 - 0.004304\,X_3X_3 - 0.005820\,X_4X_4 \\ &+ 0.04900\,X_1X_2 - 0.006075\,X_1X_3 + 0.009625\,X_1X_4 \\ &+ 0.003750\,X_2X_3 + 0.005708\,X_2X_4 - 0.001325\,X_3X_4 \end{aligned} \tag{4}$$

In the study, the effects of ultrasound-assisted extraction parameters, including temperature (X_1), time (X_2), amplitude (X_3), and propolis extract concentration (X_4) on the functional properties of yellow cherry juice were investigated in detail. The experimental results revealed that total phenolic content (TPC) varied between 256.27 mg GAE/L and 268.72 mg GAE/L, while total flavonoid content (TFC) varied between 30.20 mg CE/L and 36.31 mg CE/L. The antioxidant capacity measured by DPPH inhibition peaked at 63.26%. Meanwhile, polyphenol oxidase (PPO) activity, which plays a critical role in enzymatic browning, varied between 52.76 and 56.67%. The comparison between experimental and RSM-predicted values showed a strong correlation with minor deviations for TPC (1.64%), TFC (5.71%), DPPH (3.89%), and PPO (4.20%), demonstrating the reliability of the response surface methodology (RSM) in predicting these functional parameters. The ANOVA results in the regression model of the combination test are presented in Table 3.

X_4 had the highest effect on PPO inhibition ($F = 91.37$, $p < 0.000$), demonstrating its effectiveness in preventing enzymatic browning (Figure 1). The quadratic effects were powerful, with X_4X_4 showing the highest F values for PPO inhibition (1,986.91, $p < 0.000$) and DPPH (3,281.51, $p < 0.000$), suggesting a non-linear relationship between propolis concentration and the response. Among the tested factors, propolis extract (X_4) showed the most potent effect on both TPC ($F = 202.81$, $p < 0.000$) and TFC ($F = 558.22$, $p < 0.000$), highlighting its role in increasing the bioactive compound content of yellow cherry juice (Figures 2, 3). An increase in TFC amount was detected in thermosonication treatment. Similar increases were reported in orange juice whey (Oliveira et al., 2022) and white currant juices (Kidoń and Narasimhan, 2022) samples. The increase in ultrasound application parameters resulted in an enhanced DPPH antioxidant capacity (Figure 4). This result is consistent with the finding in the study conducted on cornelian cherry (*Cornus mas* L.) vinegar (Erdal et al., 2022). A similar result was observed in other studies where thermosonication application to purple cactus pear juice and amora (*Spondius pinnata*) juice caused an increase in antioxidant activity

TABLE 2 Measured responses used in the experimental design for RSM.

Run No.	Independent variables				Dependent variables							
	Temperature (X_1) (°C)	Time (X_2) (min)	Amplitude (X_3) (%)	Propolis extract (X_4) (mg/100 mL)	PPO (%)		TPC (mg GAE/L)		TFC (mg CE/L)		DPPH (inhibition%)	
					Experimental data	RSM predicted	Experimental data	RSM predicted	Experimental data	RSM predicted	Experimental data	RSM predicted
1	45	10	60	40	53.23	53.21	257.65	258.27	31.02	31.01	58.55	58.47
2	45	10	80	60	54.67	54.62	258.97	259.10	33.32	33.24	60.14	60.02
3	45	7	60	60	56.60	56.68	265.42	265.48	35.21	35.18	63.26	63.23
4	50	10	60	60	54.89	55.00	266.72	266.41	34.36	34.51	60.38	60.44
5	45	4	60	80	53.62	53.74	267.12	266.80	33.34	33.36	58.98	59.04
6	40	4	60	60	54.76	54.72	266.51	266.94	33.73	33.70	60.24	60.13
7	45	4	60	40	53.89	53.87	260.15	259.90	33.19	33.41	59.28	59.20
8	45	7	60	60	56.67	56.68	265.67	265.48	35.21	35.18	63.26	63.23
9	50	7	60	80	54.95	55.00	268.72	268.22	36.31	36.29	60.45	60.43
10	40	7	60	40	54.22	54.19	260.27	260.21	33.38	33.13	59.64	59.55
11	40	7	40	60	53.36	53.44	258.33	258.76	32.41	32.45	58.70	58.74
12	50	4	60	60	53.70	53.71	262.38	263.07	32.46	32.54	59.07	59.02
13	40	10	60	60	53.28	53.34	256.43	255.86	31.84	31.88	58.61	58.62
14	50	7	60	40	52.76	52.77	258.04	257.89	30.41	30.20	58.04	57.98
15	40	7	60	80	52.91	52.92	259.63	259.22	31.96	31.89	58.20	58.15
16	40	7	80	60	54.67	54.76	260.27	260.30	31.54	31.68	60.14	60.18
17	50	7	40	60	54.88	54.88	266.43	266.70	32.95	32.81	60.37	60.30
18	45	7	80	40	54.07	54.20	256.27	255.95	32.29	32.31	59.48	59.56
19	45	7	60	60	56.67	56.68	265.42	265.48	35.21	35.18	63.26	63.23
20	45	10	60	80	54.20	54.31	260.16	260.70	36.12	35.91	59.62	59.68
21	45	4	80	60	54.21	54.26	260.23	260.30	32.56	32.32	59.63	59.62
22	45	4	40	60	54.38	54.45	266.72	266.03	33.75	33.56	59.82	59.84
23	50	7	80	60	53.98	53.99	259.16	259.03	32.82	32.79	59.38	59.32
24	45	7	40	80	54.54	54.48	263.24	263.68	35.04	35.13	59.99	59.86
25	45	10	40	60	54.03	54.00	260.12	259.49	32.82	32.79	59.43	59.34
26	45	7	80	80	53.76	53.73	259.96	260.08	32.65	32.69	59.14	59.03
27	45	7	40	40	52.92	53.03	258.48	258.47	30.59	30.66	58.21	58.27
TS-YCJ	47	7.33	56.16	67.87	56.51		267.16		35.89		62.93	
Experimental values					54.23		271.61		33.95		60.48	
% Difference					4.20%		1.64%		5.71%		3.89%	

X_1, temperature; X_2, time; X_3, amplitude; X_4, propolis; TS-YCJ, thermosonicated optimized propolis yellow cherry juice; PPO, Polyphenol oxidase; TPC, total phenolic content; TFC, total flavonoid content; DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant capacity; GAE, gallic acid equivalent; CE, quercetin equivalents.

10.3389/fsufs.2025.1606602

TABLE 3 ANOVA in the regression model of the combination test.

Source	DF	PPO (%)		TPC (mg GAE/L)		TFC (mg CE/L)		DPPH (inhibition)	
		F-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-Value	*p*-value
Model	14	270.98	0.000	80.05	0.000	144.57	0.000	422.73	0.000
Linear	4	37.81	0.000	133.20	0.000	156.50	0.000	39.72	0.000
X_1	1	42.03	0.000	103.80	0.000	52.29	0.000	44.14	0.000
X_2	1	0.74	0.407	137.85	0.000	0.53	0.479	0.80	0.390
X_3	1	17.09	0.001	88.34	0.000	14.96	0.002	18.28	0.001
X_4	1	91.37	0.000	202.81	0.000	558.22	0.000	95.68	0.000
Square	4	662.97	0.000	55.51	0.000	126.53	0.000	1,180.46	0.000
X_1X_1	1	1,135.22	0.000	24.78	0.000	279.58	0.000	2,124.11	0.000
X_2X_2	1	1,026.51	0.000	23.43	0.000	93.48	0.000	1,976.49	0.000
X_3X_3	1	901.30	0.000	155.44	0.000	362.69	0.000	1,794.89	0.000
X_4X_4	1	1,986.91	0.000	136.86	0.000	175.82	0.000	3,281.51	0.000
2-Way interaction	6	165.09	0.000	60.98	0.000	148.63	0.000	172.92	0.000
X_1X_2	1	233.97	0.000	161.72	0.000	113.78	0.000	245.34	0.000
X_1X_3	1	160.30	0.000	65.97	0.000	4.34	0.059	167.61	0.000
X_1X_4	1	402.04	0.000	99.66	0.000	424.44	0.000	420.73	0.000
X_2X_3	1	21.53	0.001	22.18	0.001	22.62	0.000	22.99	0.000
X_2X_4	1	50.46	0.000	15.47	0.002	194.09	0.000	53.27	0.000
X_3X_4	1	122.25	0.000	0.89	0.364	132.51	0.000	127.57	0.000
Error	12								
Lack-of-Fit	10	5.40	0.166	18.32	0.053	*	*	*	*
Pure error	2								
Total	26								
R^2		99.68%		98.94%		99.41%		99.80%	
Adj R^2		99.32%		97.70%		98.72%		99.56%	
Pred. R^2		98.22%		93.94%		96.60%		98.83%	

X_1: temperature; X_2: time; X_3: amplitude; X_4: Propolis; DF, degree of freedom; R^2, coefficient of determination. $p < 0.05$, statistically significant; $p < 0.01$, statistically highly significant; PPO, Polyphenol oxidase; TPC, total phenolic content; TFC, total flavonoid content; DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant capacity; GAE, gallic acid equivalent; CE, quercetin equivalents, *The pure error was zero; thus, the Lack-of-Fit *F*-value and *p*-value could not be computed. This indicates that the model fits the data without detectable deviation.

values compared to heat treatment (Nayak et al., 2022). ANOVA results showed that all independent variables had a statistically significant effect on the response variables, and *p*-values below 0.05 confirmed their relevance. Temperature (X_1) played a significant role in increasing the antioxidant capacity (DPPH inhibition) ($F = 44.14$, $p < 0.000$). variables.

The regression model evaluation, based on R^2 values, showed the model's strong explanatory power. The R^2 values for PPO inhibition (99.68%), TPC (98.94%), TFC (99.41%), and DPPH inhibition (99.80%) indicated that the model explained nearly all the variations in the experimental data. These high R^2 values indicate that the model accurately represents the relationships between the independent variables and the response factors.

Moreover, the adjusted R^2 values (99.32% for PPO, 97.70% for TPC, 98.72% for TFC, and 99.56% for DPPH) confirmed that the model remained highly reliable even after adjusting the number of predictors. In addition, the discordance test results showed no significant deviation between the experimental and predicted values, further confirming the robustness of the RSM model.

In conclusion, the findings indicate that ultrasound-assisted extraction, particularly with propolis extract, significantly enhances the functional properties and stability of yellow cherry juice. Combining optimized temperature, amplitude, and propolis concentration results in improved antioxidant capacity, increased polyphenol and flavonoid content, and reduced enzymatic degradation. The exceptionally high R^2 values indicate that the developed RSM model is reliable for optimizing ultrasound-assisted extraction parameters in food processing applications. Future studies can further investigate different bioactive additives and processing parameters to maximize the' health benefits and commercial potential of functional fruit-based products.

3.2 Bioactive compounds

The results of the bioactive compounds for control yellow cherry juice, pasteurized yellow cherry juice, and thermosonicated optimized propolis yellow cherry juice are presented in Figure 5. Polyphenol

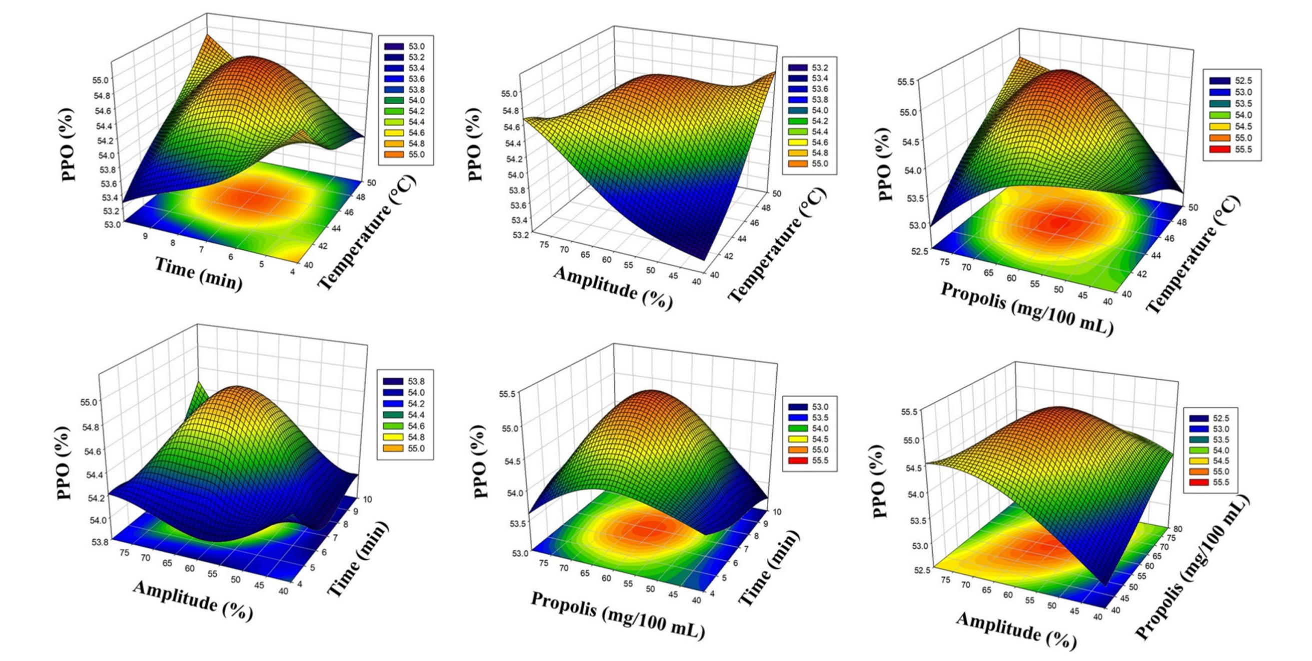

FIGURE 1
Reaction surface plots of PPO (%) as a function of significant interaction (3D).

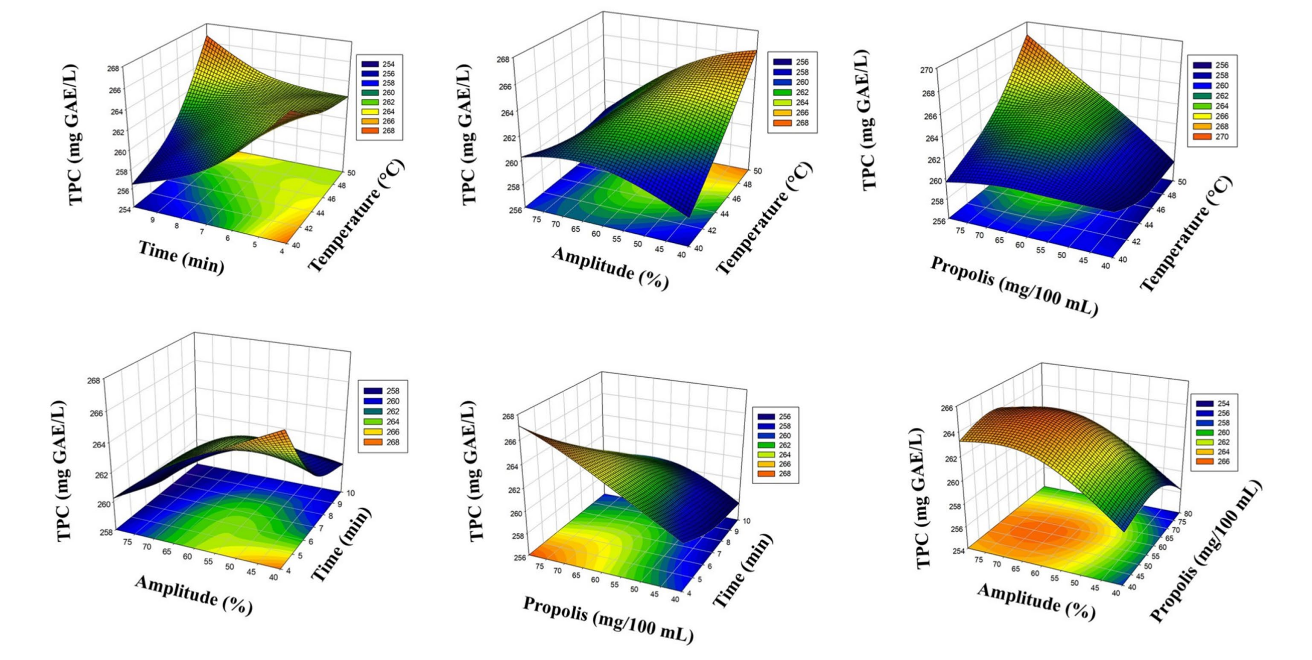

FIGURE 2
Reaction surface plots of TPC (mg GAE/L) as a function of significant interaction (3D).

oxidase activity remained stable in all treatments, C-YCJ (55.05 ± 18.72%), P-YCJ (54.09 ± 13.85%), and TS-YCJ (55.2 ± 14.30%), indicating that the enzymatic browning potential was not affected by the processing. It has been stated that sonochemistry and cavitation have an effect on enzymatic processes; these methods increase enzyme mobility, reduce diffusion resistance, make enzymes more stable in solution, affect reaction kinetics and improve mass transfer with homogeneous mixing (Villamiel et al., 2025). The extant literature posits that thermal treatment is more effective in inhibiting polyphenol oxidase, while ultrasonic treatment has been shown to increase enzyme activity (Markovinović et al., 2025). The total phenolic content (TPC) showed no significant loss, with C-YCJ at 241.4 ± 29.20 mg GAE/L, P-YCJ at 244.98 ± 32.33 mg GAE/L, and TS-YCJ at 244.63 ± 33.66 mg GAE/L. This indicates that both

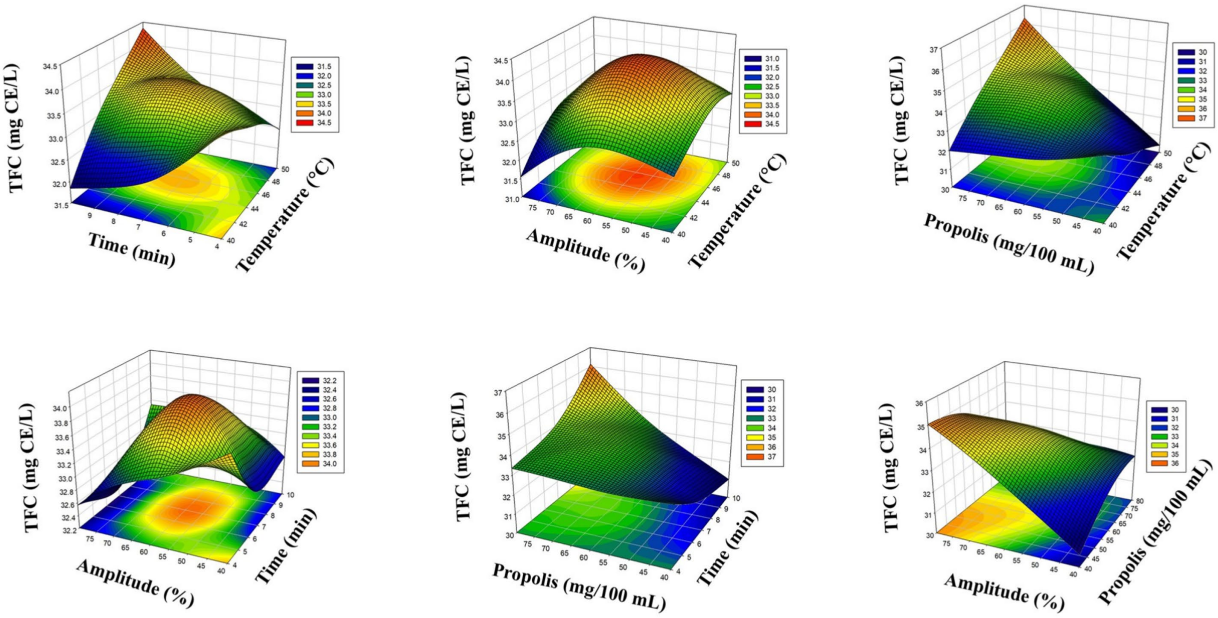

FIGURE 3
Reaction surface plots of TFC (mg CE/L) as a function of significant interaction (3D).

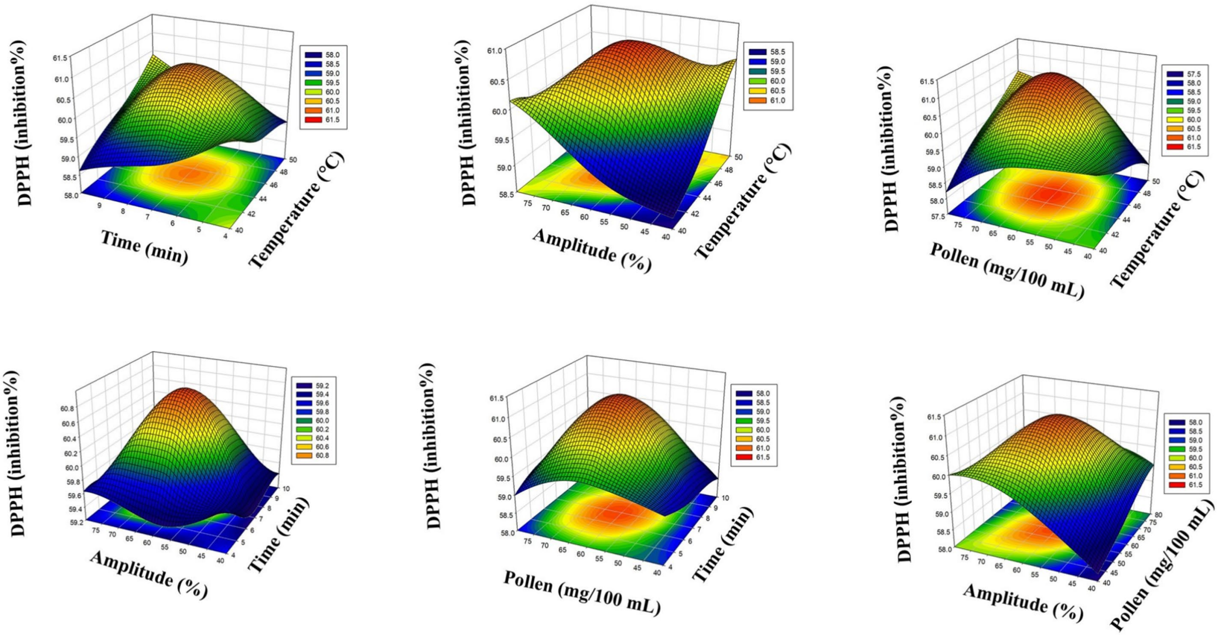

FIGURE 4
Reaction surface plots of DPPH (inhibition %) as a function of significant interaction (3D).

pasteurization and thermosonication effectively preserved the phenolic compounds. Similar to this study, Santos et al. (2024) reported that ultrasound treatment increased the TPC values of *araticum* (*Annona crassiflora*) juice (Santos et al., 2024). The observed increase in the total TPC could be attributable to the combined effect of ultrasonic cavitation and heat-induced degradation of the substrate, which results in the disruption of cells and the subsequent release of phenols that were previously bound within them (Li et al., 2025). The total flavonoid content (TFC) remained statistically unchanged (C-YCJ: 29.26 ± 4.95 mg CE/L, P-YCJ: 28.07 ± 4.86 mg CE/L, TS-YCJ: 28.56 ± 5.92 mg CE/L) ($p > 0.05$). Antioxidant capacity measured by DPPH radical inhibition showed a slight but non-significant improvement in the thermosonicated sample (TS-YCJ: 57.06% ± 2.98%) compared to the control (C-YCJ: 56.59% ± 6.74%) and pasteurized fruit juice (P-YCJ: 54.28% ± 4.04%). These results emphasize that thermosonication effectively preserves bioactive

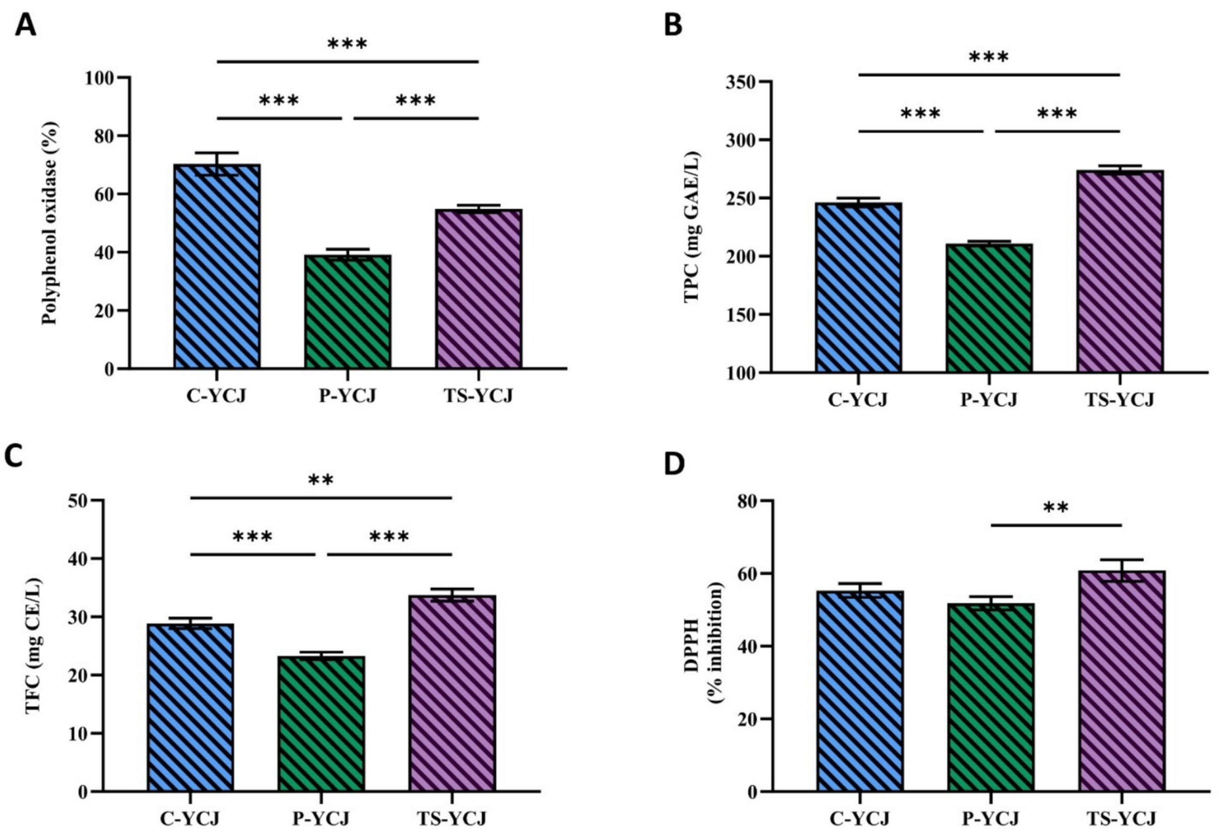

FIGURE 5
Bioactive compounds results of C-YCJ, P-YCJ, and TS-YCJ samples. The results are the mean ± standard deviation (n = 3). Numbers above bars indicate statistically significant differences (**p < 0.01. ***p < 0.001). Polyphenol oxidase **(A)**, TPC **(B)**, TFC **(C)**, and DPPH **(D)** results of C-YCJ, P-YCJ, and TS-YCJ samples. PPO, Polyphenol oxidase; TPC, total phenolic content; TFC, total flavonoi. C-YCJ, Control yellow cherry juice; P-YCJ, Thermal pasteurized yellow cherry juice; TS-YCJ, Thermosonication-treated yellow cherry juice; PPO, Polyphenol oxidase; TPC, total phenolic content; TFC, total flavonoid content; DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant capacity; GAE, gallic acid equivalent; CE, quercetin equivalents.

compounds, making it a promising alternative to thermal processing. Parallel to our study, Shaik and Chakraborty (2024) found that ultrasound-treated Sweet Lime Juice had significantly higher total antioxidant activity and lower PPO enzyme activity than the pasteurized sample (Shaik and Chakraborty, 2024). In addition, it has been posited by the researchers that ultrasound has a stimulating effect on antioxidant content and that it increases the processes involved in the production of antioxidants in fruit juices (Noorisefat et al., 2025).

Pearson correlation coefficients for bioactive compounds, amino acids, and sensory properties are given in Figure 6. Positive or negative correlation coefficients indicate a direct or inverse relationship between the parameters. Significant correlation differences are observed among the three samples: control yellow cherry juice pasteurized yellow cherry juice and thermosonicated optimized propolis yellow cherry juice Especially, the correlation between sensory properties and functional components such as TPC, TFC, and antioxidant capacity (DPPH) varied among the samples. While a moderate correlation was observed between total phenolic and flavonoid substance content and antioxidant capacity in the control sample ($r \approx 0.60$), this correlation became more potent in the ultrasound-treated sample ($r > 0.80$). This indicates that ultrasound treatment enhances the extraction of phenolic compounds by breaking down cell walls, thereby directly increasing the antioxidant capacity. In addition, the correlation is relatively weaker in the pasteurized sample ($r \approx 0.40$). This result indicates that heat treatment causes partial degradation of phenolic compounds, limiting the antioxidant capacity.

3.3 Physicochemical parameters

The pH values remained stable in all treatments, and no statistically significant difference ($p > 0.05$) was observed among C-YCJ (control yellow cherry juice) (3.68 ± 0.02), P-YCJ (pasteurized yellow cherry juice) (3.67 ± 0.02), and TS-YCJ (thermosonicated optimized propolis yellow cherry juice) (3.71 ± 0.02). This stability indicates that neither pasteurization nor thermosonication resulted in any significant change in acidity. While a slight decrease in the pH value of yellow cherry juice was observed after the pasteurization process, no significant change in pH value was detected after thermosonication. Similar results regarding pH were reported in studies conducted on pasteurized grape juice samples (Ergezer et al., 2018), yellow and red watermelon juice samples (Yıkmış, 2020), thermosonicated purple cactus pear juices (Cruz-Cansino et al., 2015), carrot juice (Jabbar et al., 2015), black mulberry juice (Dinçer and Topuz, 2015) and apple juice (Abid et al., 2014). A significant increase ($p < 0.05$) in total soluble solids (TSS, °Brix) was recorded in TS-YCJ

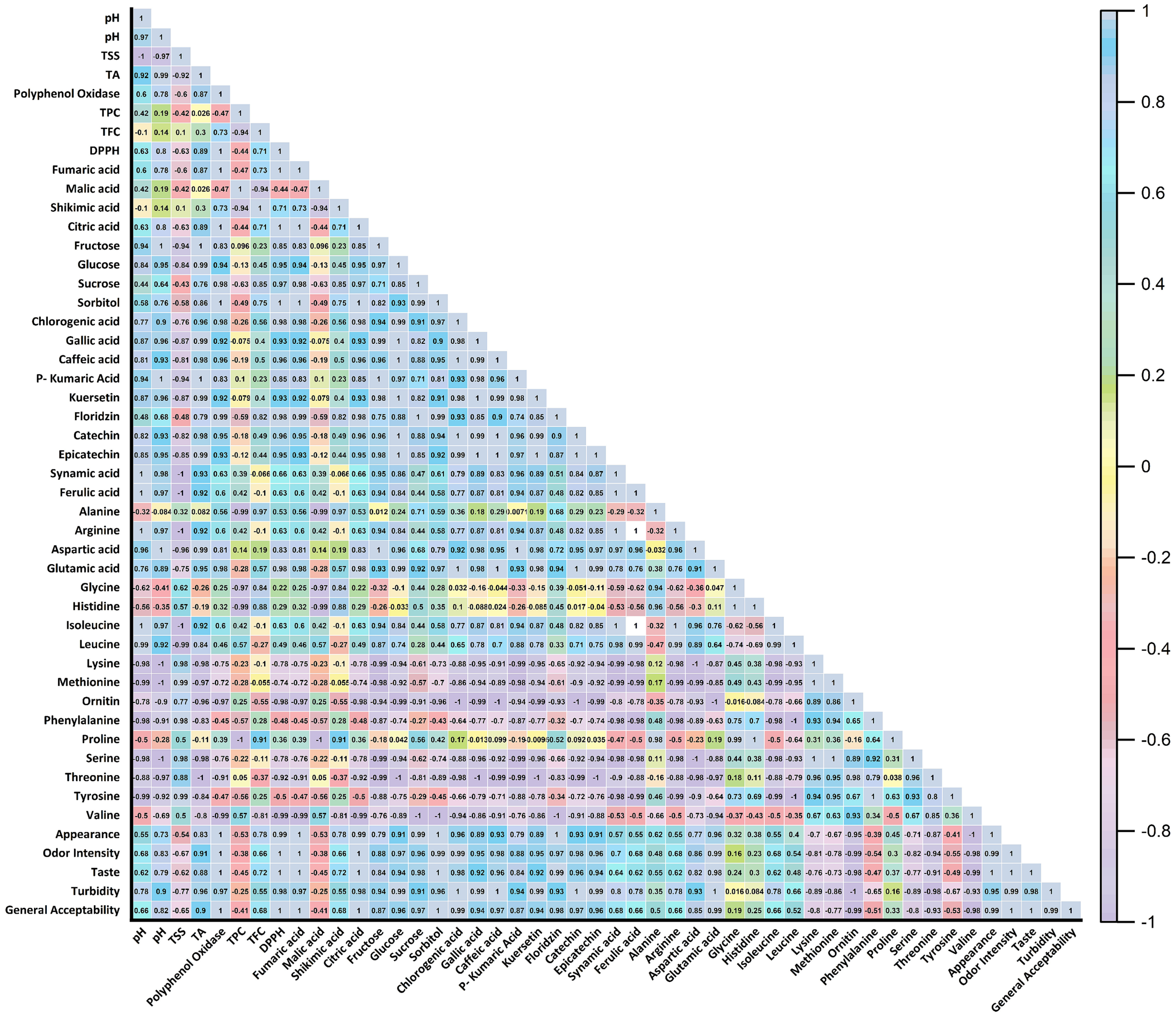

FIGURE 6
Pearson correlation coefficients for bioactive compounds, amino acids, and sensory properties.

(14.68 ± 0.03). This increase is probably due to water loss or increased dissolution of solids during thermosonication. The highest pH value was observed in the TS-YCJ sample. Sasikumar and Jaiswal (2022) investigated the effect of thermosonication on the physicochemical properties of blood fruit juice. Exposure of blood fruit juice to different temperature and time combinations of TS did not show significant changes in the pH of the blood fruit drink. However, as the thermosonication temperature increased, a rise in pH was observed, which may be attributed to the deterioration of heat-sensitive foods (Sasikumar and Jaiswal, 2022). When titratable acidity (TA) was compared among the samples, C-YCJ (5.97 ± 0.06 g/L), P-YCJ (5.95 ± 0.04 g/L), and TS-YCJ (6.00 ± 0.04 g/L) showed no significant difference ($p > 0.05$). These results indicate that thermosonication and pasteurization did not alter the acid–base balance of the juice, preserving its natural taste profile. However, the increase in TSS with thermosonication suggests a potential improvement in sweetness perception, which may increase consumer acceptance. Figure 7 shows the physicochemical parameter results of C-YCJ, P-YCJ and TS-YCJ samples.

3.4 Analysis of amino acids

Primary metabolites, such as amino acids, sugars, and organic acids, as well as fruit-specific secondary metabolites, possess unique chemical structures that play a crucial role in the general functions of cells and contribute to the aroma and taste of fruit juices (Sobolev et al., 2015). The analysis of amino acid profiles and contents can provide significant insights into various factors, including quality, botanical origin, and nutritional content, of fruits and fruit-derived foods (Bilgin et al., 2019). The results of the amino acid analysis of C-YCJ, P-YCJ, and TS-YCJ are given in Table 4. The amino acid composition of yellow cherry juice (YCJ) was significantly affected by the applied processing methods, as observed in control (C-YCJ), pasteurized (P-YCJ), and thermosonicated (TS-YCJ) samples. The total amino acid content was highest in C-YCJ (2.09 ± 1.48 mg/100 g), followed by P-YCJ (1.31 ± 0.92 mg/100 g). There was a statistically significant decrease ($p < 0.05$) in TS-YCJ (0.01 ± 0.01 mg/100 g). This significant decrease in the thermosonicated sample suggests that the effects of heat and cavitation during processing may cause degradation

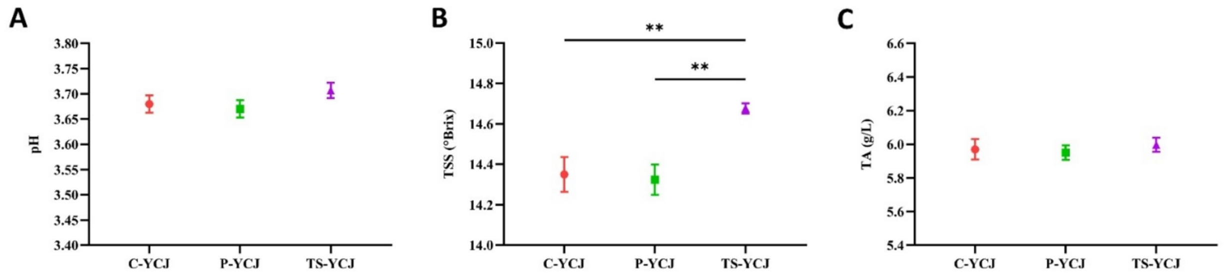

FIGURE 7
Physicochemical parameter results of C-YCJ, P-YCJ, and TS-YCJ samples. pH **(A)**, TSS **(B)**, and TA **(C)** results of C-YCJ, P-YCJ, and TS-YCJ samples.

TABLE 4 C-YCJ, P-YCJ and TS-YCJ amino acid analysis results.

Compounds No.	Amino acids (mg/100 g)	C-YCJ	P-YCJ	TS-YCJ
1	Alanine	0.45 ± 0.32^{b}	0.16 ± 0.12^{b}	0.22 ± 0.16^{a}
2	Arginine	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.03 ± 0.02^{b}
3	Aspartic acid	0.22 ± 0.15^{c}	0.08 ± 0.06^{b}	0.55 ± 0.39^{a}
4	Cystine	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	Glutamic acid	0.35 ± 0.25^{c}	0.25 ± 0.18^{b}	0.40 ± 0.28^{a}
6	Glycine	0.27 ± 0.19^{b}	0.16 ± 0.11^{a}	0.14 ± 0.10^{ab}
7	Histidine	0.16 ± 0.12^{b}	0.05 ± 0.03^{a}	0.04 ± 0.02^{c}
8	Isoleucine	0.01 ± 0.01^{b}	0.01 ± 0.01^{a}	0.04 ± 0.03^{b}
9	Leucine	0.04 ± 0.02^{b}	0.09 ± 0.07^{a}	0.32 ± 0.23^{c}
10	Lysine	0.40 ± 0.28^{b}	0.49 ± 0.34^{a}	0.07 ± 0.05^{c}
11	Methionine	0.06 ± 0.04^{c}	0.07 ± 0.05^{a}	0.01 ± 0.01^{b}
12	Ornitin	0.83 ± 0.58^{b}	1.45 ± 1.03^{a}	0.48 ± 0.34^{c}
13	Phenylalanine	0.17 ± 0.12^{b}	0.14 ± 0.10^{a}	0.01 ± 0.01^{c}
14	Proline	0.07 ± 0.05^{b}	0.01 ± 0.01^{a}	0.01 ± 0.00^{c}
15	Serine	0.10 ± 0.07^{b}	0.12 ± 0.09^{a}	0.03 ± 0.02^{c}
16	Threonine	0.14 ± 0.10^{b}	0.21 ± 0.15^{a}	0.06 ± 0.04^{c}
17	Tyrosine	0.18 ± 0.13^{b}	0.16 ± 0.12^{a}	0.06 ± 0.04^{c}
18	Valine	0.01 ± 0.00^{b}	0.04 ± 0.03^{a}	0.01 ± 0.01^{c}
19	Taurine	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Total	2.09 ± 1.48^{c}	1.31 ± 0.92^{a}	0.01 ± 0.01^{b}

The results are the mean ± standard deviation (n = 3). The values marked with different letters within the line are significantly different from each other ($p < 0.05$). C-YCJ, Control yellow cherry juice; P-YCJ, Thermal pasteurized yellow cherry juice; TS-YCJ, Thermosonication-treated yellow cherry juice.

or structural changes to amino acids, rendering them undetectable or significantly reduced. Essential amino acids, such as lysine, leucine, and valine, showed the highest concentrations in P-YCJ, with lysine being the most notable at 0.49 ± 0.34 mg/100 g, significantly higher than in C-YCJ (0.4 ± 0.28 mg/100 g) and TS-YCJ (0.07 ± 0.05 mg/100 g). The observed differences were statistically significant ($p < 0.05$), indicating that pasteurization had a protective effect on essential amino acids, whereas thermosonication led to significant losses. Among the nonessential amino acids, glutamic acid, which contributes to palatability, was significantly higher in TS-YCJ (0.4 ± 0.28 mg/100 g) than in P-YCJ (0.25 ± 0.18 mg/100 g) and C-YCJ (0.35 ± 0.25 mg/100 g). However, ornithine, which plays a role in nitrogen metabolism, was best retained in P-YCJ (1.45 ± 1.03 mg/100 g), significantly higher than in C-YCJ (0.83 ± 0.58 mg/100 g) and TS-YCJ (0.48 ± 0.34 mg/100 g). These statistically significant differences highlight how processing conditions differentially affect certain amino acids and that pasteurization is the more effective method for preserving amino acid integrity. Erdal et al. (2022) reported that thermosonication application led to a decrease in aspartic acid and glutamic acid levels. In contrast, our study observed the opposite, with an increase in these results (Erdal et al., 2022). From a statistical perspective, the presence of different superscripts in the data indicates that the differences between the processing methods are statistically significant ($p < 0.05$), confirming that thermal and non-thermal treatments have distinct effects on amino acid stability. Amino acids function as precursors of volatile compounds, thereby influencing the aroma of juice (Hendriks, 2018). Especially phenylalanine, valine, leucine, isoleucine, and play a role in determining the aroma profile. The significant decrease in total amino acids in TS-YCJ suggests that thermosonication is not a suitable method for preserving amino acids in juices unless the processing parameters are optimized. The formation of free radicals during acoustic cavitation can have a negative effect on the stability of bioactive ingredients, as OH radicals can cause degradation (Sun et al., 2016). relatively high standard deviations in some amino acid measurements, particularly for ornithine and lysine, suggest variability in retention levels, likely due to differences in the juice matrix or interactions between amino acids and processing conditions. Pasteurization has become a preferred method to preserve nutritional quality while ensuring microbial safety, demonstrating that essential amino acids are best preserved. The results suggest that changes in amino acid profiles resulting from processing should be taken into account when designing food preservation strategies. Future research should focus on optimizing thermosonication parameters, such as power density and duration, to enhance amino acid retention while preserving the functional benefits of this new technology.

Correlations between amino acid content, phenolic compounds, and sensory properties are also remarkable (Figure 6). Mainly, positive correlations were observed between amino acids such as arginine ($r \approx +0.68$), histidine ($r \approx +0.63$), and glutamic acid ($r \approx +0.75$) and total phenolic substance and antioxidant capacity. This suggests that the presence of phenolic compounds has a direct impact on the functional quality of the product in conjunction with amino acids. In addition, high positive correlations were observed between glutamic acid and histidine with taste ($r \approx +0.70$) and general acceptability ($r \approx +0.68$). Glutamic acid contributed to the umami taste profile, while histidine and arginine created a complex flavor profile in the product, positively affecting sensory acceptance. This relationship was relatively weaker in the control and pasteurized samples, with a correlation coefficient of approximately $r \approx +0.40$. In addition, the positive correlation between amino acids such as tyrosine ($r \approx +0.65$) and serine ($r \approx +0.60$) and total phenolic content and antioxidant capacity confirms the effect of bioactive compounds on sensory quality. The correlation between amino acids, such as proline and ornithine, and antioxidant capacity and sensory properties was weaker, with a correlation coefficient of approximately +0.40. These results suggest that the synergistic relationship between phenolic compounds and amino acids directly affects both the bioactive and sensory qualities of the product.

3.5 Organic acid profile

The organic acid results of C-YCJ, P-YCJ, and TS-YCJ are given in Table 5. The organic acid composition plays a crucial role in determining the sensory and nutritional profiles of fruit juices. In this study, total organic acid content was significantly different among the treatments ($p < 0.05$), with the highest concentration observed in thermosonicated juice (TS-YCJ: 1,177.96 ± 18.87 mg/L), followed by control juice (C-YCJ: 1,158.53 ± 40.78 mg/L) and the lowest concentration in pasteurized juice (P-YCJ: 1,081.79 ± 39.41 mg/L). This indicates that pasteurization significantly decreased the organic acid content, likely due to the thermal degradation or volatilization of certain acid compounds. Conversely, thermosonication helped preserve or even increase the levels of organic acids, as it facilitated better extraction from cellular structures without significant heat-induced losses. Among individual organic acids, malic acid was the dominant compound in all samples, with the highest concentration observed in TS-YCJ (1,174.38 ± 19.02 mg/L), which was significantly higher ($p < 0.05$) than in C-YCJ (1,135.1 ± 41.64 mg/L) and P-YCJ (1,078.34 ± 39.56 mg/L). This trend suggests that thermosonication improved the retention of malic acid, which is known for increasing the sourness and stability of fruit juice. Citric acid, another important organic acid affecting flavor, followed a similar trend, having a significantly higher ($p < 0.05$) concentration in TS-YCJ (20.69 ± 1.24 mg/L) compared to C-YCJ (19.98 ± 0.79 mg/L) and P-YCJ (18.45 ± 0.41 mg/L). In a study conducted by Silva et al. (2020), it was observed that applying high-intensity ultrasound to orange juice enriched with xylooligosaccharides increased the amounts of malic acid and citric acid to a nominal power of 600 W. Still, the concentrations of malic acid and citric acid decreased as the nominal power increased. They explained this initial increase by the mechanical rupture of intracellular structures such as cell walls and plastids due to acoustic cavitation (Silva et al., 2020). Shikimic acid and fumaric

TABLE 5 Organic acid, sugar, and phenolic compounds analysis results of C-YCJ, P-YCJ, and TS-YCJ.

Studied compound		Samples		
		C-YCJ	P-YCJ	TS-YCJ
Organic acids (g/100 mL)	Fumaric acid	0.44 ± 0.04[a]	0.40 ± 0.01[a]	0.45 ± 0.03[a]
	Malic acid	1,135.10 ± 41.64[a]	1,078.34 ± 39.56[a]	1,174.38 ± 19.02[a]
	Shikimic acid	3.03 ± 0.11[a]	3.05 ± 0.16[a]	3.14 ± 0.18[a]
	Citric acid	19.98 ± 0.79[a]	18.45 ± 0.41[a]	20.69 ± 1.24[a]
	Total	1,158.53 ± 40.78[a]	1,081.79 ± 39.41[a]	1,177.96 ± 18.87[a]
Sugars (g/L)	Fructose	50.61 ± 2.91[a]	48.68 ± 2.20[a]	54.39 ± 2.52[a]
	Glucose	66.49 ± 2.73[a]	62.77 ± 2.99[a]	69.70 ± 2.74[a]
	Sucrose	14.91 ± 1.44[a]	13.79 ± 1.44[a]	14.82 ± 0.59[a]
	Sorbitol	11.99 ± 0.78[a]	10.79 ± 1.03[a]	12.13 ± 1.92[a]
	Total	137.72 ± 1.00[ab]	130.99 ± 1.51[a]	145.65 ± 3.69[b]
Phenolic compounds (µg/mL)	Klorogenic acid	36.86 ± 0.93[ab]	33.50 ± 0.92[a]	38.64 ± 0.54[b]
	Gallic acid	4.68 ± 0.04[b]	4.26 ± 0.04[a]	5.12 ± 0.09[c]
	Caffeic acid	1.86 ± 0.03[b]	1.69 ± 0.04[a]	1.98 ± 0.04[b]
	p-Coumaric acid	0.71 ± 0.03[a]	0.65 ± 0.02[a]	0.83 ± 0.03[b]
	Quercetin	6.63 ± 0.10[b]	6.03 ± 0.11[a]	7.25 ± 0.11[c]
	Phloridzin	4.62 ± 0.07[b]	4.20 ± 0.06[a]	4.61 ± 0.07[b]
	Catechin	6.67 ± 0.15[ab]	6.02 ± 0.21[a]	7.14 ± 0.16[b]
	Epicatechin	3.08 ± 0.07[a]	2.82 ± 0.04[a]	3.31 ± 0.23[a]
	Cinnamic acid	0.21 ± 0.01[a]	0.20 ± 0.01[a]	0.45 ± 0.04[b]
	Ferulic acid	0.02 ± 0.00[a]	0.02 ± 0.01[a]	0.12 ± 0.01[b]
	Total	65.11 ± 1.28[b]	59.15 ± 1.31[a]	69.37 ± 1.18[b]

The results are the mean ± standard deviation ($n = 3$). The values marked with different letters within the line are significantly different from each other ($p < 0.05$). C-YCJ, Control yellow cherry juice; P-YCJ, Thermal pasteurized yellow cherry juice; TS-YCJ, Thermosonication-treated yellow cherry juice.

acid levels were not significantly affected by processing ($p > 0.05$), indicating their relative stability under thermal and non-thermal conditions. The significant reduction in total organic acid content in pasteurized samples compared to thermosonication and control juices confirms that heat treatment negatively affects acid retention. In contrast, thermosonication offers a superior method for preserving these bioactive compounds.

3.6 Sugar profile

The sugar analysis results of C-YCJ, P-YCJ, and TS-YCJ are given in Table 5. Sugars contribute significantly to the sweetness and overall sensory profile of fruit juices. Total sugar content showed significant differences among treatments ($p < 0.05$), with the highest concentration observed in thermosonicated juice (TS-YCJ: 145.65 ± 3.69 g/L), followed by control juice (C-YCJ: 137.72 ± 1.00 g/L) and the lowest concentration in pasteurized juice (P-YCJ: 130.99 ± 1.51 g/L). The significant decrease in total sugar content in P-YCJ suggests that pasteurization may lead to sugar degradation or losses associated with the Maillard reaction. At the same time, thermosonication may potentially increase or decrease sugar retention in fruit matrix structures. Among individual sugars, fructose, and glucose were the dominant sugars in all samples. The fructose content was highest in TS-YCJ (54.39 ± 2.52 g/L), followed by C-YCJ (50.61 ± 2.91 g/L), and was significantly lower in P-YCJ (48.68 ± 2.2 g/L) ($p < 0.05$). A similar trend was observed for glucose, with TS-YCJ (69.7 ± 2.74 g/L) showing the highest levels compared to C-YCJ (66.49 ± 2.73 g/L) and P-YCJ (62.77 ± 2.99 g/L). These results suggest that thermosonication promotes better sugar retention or removal, likely due to enhanced disruption of cell walls and facilitation of intracellular sugar release. In the study conducted by Liao et al. (2020), it was found that the amount of glucose and fructose increased due to thermosonication treatment of clear red pitaya juice, similar to our study (Dadan et al., 2022). Ultrasound treatment provides greater penetration of the solvent into the sample matrix by applying mechanical effects through shear forces (Özdemir et al., 2022). Sucrose and sorbitol contents remained statistically unchanged between treatments ($p > 0.05$), sucrose levels varied between 13.79 ± 1.44 g/L (P-YCJ) and 14.91 ± 1.44 g/L (C-YCJ), and sorbitol levels varied between 10.79 ± 1.03 g/L (P-YCJ) and 12.13 ± 1.92 g/L (TS-YCJ). These findings indicate that thermosonication does not significantly alter sucrose and sorbitol levels, but effectively increases the retention of monosaccharides, such as fructose and glucose, which are crucial for preserving natural sweetness. Overall, these results suggest that while pasteurization may lead to sugar losses, thermosonication helps retain or remove more sugar, potentially improving the sensory properties of yellow cherry juice. This suggests that thermosonication may be a preferred non-thermal processing method to preserve the natural sweetness of juices while maintaining microbiological safety. It has been demonstrated in the existing literature on the subject that ultrasound can instigate a range of reactions through the generation of hydroxyl radicals. Furthermore, it has been evidenced that ultrasound can enhance polymerization/depolymerization reactions and improve diffusion rates, amongst other effects (Sun et al., 2016).

When the Pearson correlation graph is examined, the distribution between sugar components and sensory acceptance varies among the differences (Figure 6). While the distribution between fructose and glucose, as well as taste and general acceptability, in the control sample remained at a moderate level ($r \approx +0.50$), it became quite strong in the sample treated with ultrasound ($r \approx +0.70$). In this case, it is evident that structures heated with ultrasound facilitate disintegration, leading to a more efficient extraction of sugar components and thereby enhancing the perception of sweetness. In the pasteurized sample, the improvement in fructose and glucose content, as well as sensory acceptance, is at a lower level ($r \approx +0.30$). This situation indicates that the losses in sugar during pasteurization are minimal in terms of taste and general acceptability. In general, the differences between the three signals reveal that ultrasound has a positive impact on bioactive programs and auditory quality, while pasteurization shows a limitation in this efficiency.

3.7 Phenolic compounds profile

The results of C-YCJ, P-YCJ, and TS-YCJ phenolic compound analysis are given in Table 5. Phenolic compounds are the main bioactive components that contribute to the antioxidant properties, stability, and health benefits of fruit juices. Phenolic compounds are secondary plant metabolites consisting of aromatic rings attached to one or more hydroxyl groups (Yusoff et al., 2022). Total phenolic content (sum of individual phenolics) differed significantly among treatments ($p < 0.05$), with the highest concentration in thermosonicated juice (TS-YCJ: 69.37 ± 1.18 μg/mL), followed by control juice (C-YCJ: 65.11 ± 1.28 μg/mL) and the lowest concentration in pasteurized juice (P-YCJ: 59.15 ± 1.31 μg/mL). This decrease in P-YCJ suggests that thermal treatment probably led to phenolic degradation due to oxidation and heat-induced structural changes. In contrast, thermosonication appeared to maintain or even improve the phenolic profile by minimizing oxidation while promoting the release of bound phenolics from cell walls. Among individual phenolic compounds, chlorogenic acid, quercetin, and catechin showed significant changes among treatments. Chlorogenic acid content was significantly higher in TS-YCJ (38.64 ± 0.54 μg/mL) compared to C-YCJ (36.86 ± 0.93 μg/mL, $p < 0.05$) and P-YCJ (33.5 ± 0.92 μg/mL, $p < 0.01$), indicating that thermosonication improved chlorogenic acid retention, probably through improved solubilization or extraction efficiency. Similarly, quercetin levels were highest in TS-YCJ (7.25 ± 0.11 μg/mL), which was significantly higher than C-YCJ (6.63 ± 0.1 μg/mL, $p < 0.05$) and P-YCJ (6.03 ± 0.11 μg/mL, $p < 0.01$), confirming that thermosonication better-preserved flavonoid compounds compared to pasteurization. Ultrasound application increased the amount of p-coumaric acid, caffeic acid, and chlorogenic acid, which are phenolic compounds, in hawthorn vinegar. Contrary to our study's results, Dadan et al. (2022) found no significant difference in the amounts of p-coumaric acid and chlorogenic acid in blue honeysuckle (*Lonicera caerulea L.*) (Dadan et al., 2022). In our study, it was concluded that yellow cherry is rich in caffeic acid. Similar results to those of our study were reported by Özdemir et al. (2022), who also presented findings from their research on hawthorn vinegar (Özdemir et al., 2022).

The content of catechin, an important flavonol with strong antioxidant activity, followed the same trend and significantly exceeded TS-YCJ (7.14 ± 0.16 μg/mL), C-YCJ (6.67 ± 0.15 μg/mL, $p < 0.05$), and P-YCJ (6.02 ± 0.21 μg/mL, $p < 0.01$). This suggests that thermal treatment degrades catechin, while thermosonication prevents its loss or facilitates its release. Among the treatments, significant differences were observed in the concentrations of gallic acid, caffeic acid, p-coumaric acid, ferulic acid, and cinnamic acid. The gallic acid content was highest in TS-YCJ (5.12 ± 0.09 μg/mL) and showed a significant increase compared to C-YCJ (4.68 ± 0.04 μg/mL, $p < 0.05$) and P-YCJ (4.26 ± 0.04 μg/mL, $p < 0.01$), indicating that thermosonication protected hydroxybenzoic acids, such as gallic acid, which are highly sensitive to thermal degradation. Caffeic acid levels were also higher in TS-YCJ (1.98 ± 0.04 μg/mL) compared to C-YCJ (1.86 ± 0.03 μg/mL, $p > 0.05$) and significantly higher than P-YCJ (1.69 ± 0.04 μg/mL, $p < 0.01$). p-Coumaric acid content was increased considerably in TS-YCJ (0.83 ± 0.03 μg/mL) compared to C-YCJ (0.71 ± 0.03 μg/mL, $p < 0.05$) and P-YCJ (0.65 ± 0.02 μg/mL, $p < 0.01$), further supporting the potential of thermosonication to improve the retention of hydroxycinnamic acids. In a study conducted by Margean et al. (2020), red grape juice was subjected to thermal pasteurization and ultrasonic treatment. The results showed that ultrasonic treatment gave more positive results regarding polyphenol content (Margean et al., 2020). This finding is consistent with our study, indicating that ultrasonic treatment may be more effective in preserving or increasing polyphenols. In particular, ferulic acid and cinnamic acid showed the most significant increases in TS-YCJ, and ferulic acid increased to 0.12 ± 0.01 μg/mL compared to P-YCJ (0.02 ± 0.01 μg/mL, $p < 0.001$) and C-YCJ (0.02 ± 0 μg/mL, $p < 0.001$). Similarly, cinnamic acid content was significantly higher in TS-YCJ (0.45 ± 0.04 μg/mL) compared to P-YCJ (0.2 ± 0.01 μg/mL, $p < 0.001$) and C-YCJ (0.21 ± 0.01 μg/mL, $p < 0.01$). These findings suggest that thermosonication enhances the availability of bound phenolic acids, likely by degrading the plant matrix and thereby improving extractability. The high positive correlation between DPPH inhibition and individual phenolic compounds such as gallic acid (r = +0.85), chlorogenic acid (r = +0.78) and caffeic acid (r = +0.82) supports the critical role of phenolic compounds in antioxidant activity (Figure 6). These results suggest that ultrasound application is effective in preserving phenolic compounds and enhancing their antioxidant capacity.

In general, pasteurization resulted in significant losses of phenolic compounds, likely due to oxidation and degradation, whereas thermosonication helped preserve or improve these bioactive components. The substantial retention in chlorogenic acid, catechin, and quercetin in TS-YCJ suggests that thermosonication effectively protects flavonoids and phenolic acids from degradation, likely by minimizing heat exposure and enhancing mass transfer. The increase in ferulic and cinnamic acids suggests that thermosonication may improve the functional properties of yellow cherry juice by increasing the release of bound phenolics. The increased presence of phenolic compounds has been attributed to their release from cell walls, resulting from the disruption of these cell walls due to cavitation phenomena that occurred during the sonication process (Maia et al., 2025). These results confirm that thermosonication is a superior alternative to pasteurization for preserving and enhancing the phenolic composition of juices, which may contribute to improved antioxidant capacity, enhanced health benefits, and increased consumer acceptance.

3.8 Sensory properties

The sensory analysis results of C-YCJ, P-YCJ, and TS-YCJ are presented in Figure 8. The sensory properties of yellow cherry juice (YCJ) were significantly affected by the applied processing

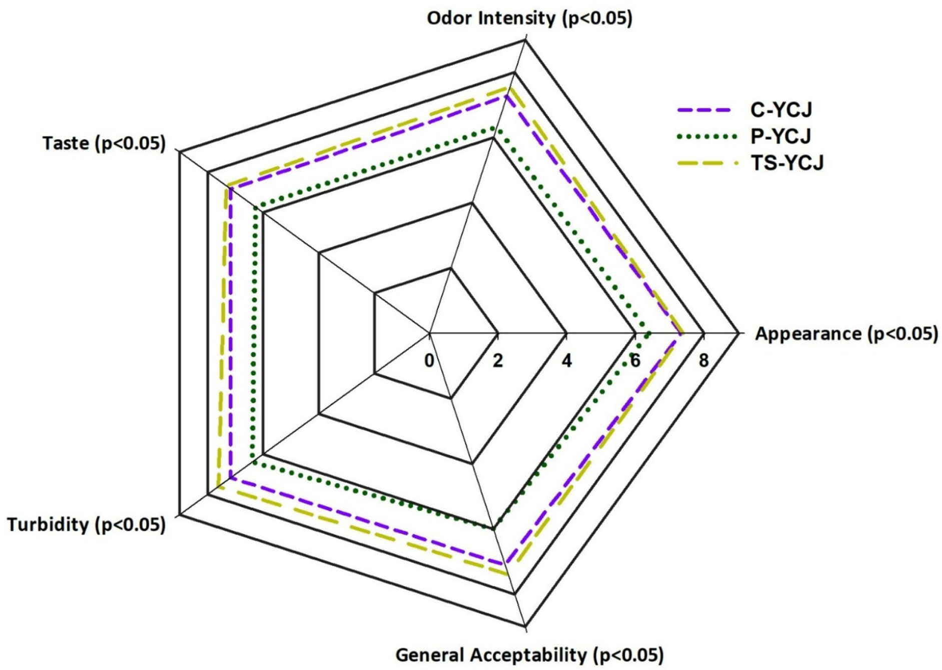

FIGURE 8
Sensory analysis results of C-YCJ, P-YCJ, and TS-YCJ.

methods, as shown by the results obtained for control (C-YCJ), pasteurized (P-YCJ), and thermosonicated (TS-YCJ) samples. For all sensory attributes evaluated (color, aroma, taste, turbidity, and overall acceptability), pasteurized YCJ (P-YCJ) had the lowest scores, while both control and thermosonicated samples performed significantly better ($p < 0.05$). The color score of TS-YCJ (7.39 ± 0.78) was slightly above that of C-YCJ (7.33 ± 0.69) and significantly better than that of P-YCJ (6.39 ± 0.92), suggesting that thermosonication may help preserve the visual appeal of water. A similar trend was observed for aroma, where TS-YCJ (7.56 ± 0.7) had the highest score, followed by C-YCJ (7.28 ± 0.75); the lowest score was recorded for P-YCJ (6.33 ± 0.84). This finding is consistent with the results reported by Guo et al. (2025) in a study on the effects of ultrasound on tomato juice (Guo et al., 2025). Paralel present study, Mizuta et al. (2025) found that there was no significant difference in aromo parameter ($p < 0.05$) between the control strawberry dairy beverage sample and the samples treated ultrasound (Mizuta et al., 2025). This suggests that thermal pasteurization may lead to the loss of volatile aromatic compounds, while thermosonication may help preserve or even increase them. Taste scores followed a similar pattern, with TS-YCJ (7.33 ± 0.59) and C-YCJ (7.17 ± 1.15) scoring significantly higher than P-YCJ (6.28 ± 1.02), implying that pasteurization may have caused thermal degradation of flavor-related compounds. In a study conducted with cashew apple juice, no significant difference was found between the sensory properties of the thermosonicated sample and the control samples (Deli et al., 2022). In a study where pasteurization and thermosonication processes were applied to quince juice, Yıkmış et al. (2019) reported that there was no significant difference in color and general acceptability evaluation, while no significant difference was found between the two processes (Yıkmış et al., 2019). Turbidity and general acceptability exhibited a statistically significant distribution, reinforcing the notion that thermosonication enhances specific sensory properties. The turbidity score was the highest in TS-YCJ (7.61 ± 0.7), followed by C-YCJ (7.17 ± 0.92); the lowest score was observed in P-YCJ (6.39 ± 1.14). This may be related to changes in suspended particles or colloidal stability due to different processing techniques. Overall acceptability scores were consistent with these findings; TS-YCJ (7.39 ± 0.92) and C-YCJ (7.11 ± 1.08) received significantly better evaluations than P-YCJ (6.0 ± 1.03), confirming that pasteurization harms consumer preference. Statistically, the presence of different superscripts in each category ($p < 0.05$) confirms that the differences between treatments are significant. The relatively high standard deviations, especially for taste and overall acceptability, indicate some variability in panelists' preferences, probably due to individual sensitivity to changes in aroma and flavor. These findings suggest that thermosonication is a promising alternative to pasteurization, as it preserves or even enhances sensory properties while mitigating the negative effects associated with traditional heat treatments. Anaya-Esparza et al. (2017) also reported that thermosonication treatment in fruit juices can effectively enhance enzymatic and microbial inactivation without compromising quality attributes (Anaya-Esparza et al., 2017). Future studies can further investigate consumer perception and optimize thermosonication parameters to maximize both sensory quality and microbial safety. Pearson's positive correlation coefficients among the taste was significantly correlated with PPO (1), DPPH (1), fumaric acid (1), citric acid (1), and sorbitol (1). The appearance showed a significant negative correlation with ornithine (−0.95) and threonine (−0.87) (Figure 6).

3.9 Principal component analysis (PCA)

The PCA plot impressively illustrates the differences in chemical and sensory properties of yellow cherry juice after various processing methods (control, pasteurization, and thermosonication). The plot exhibits a high total variance explanation rate (100%), with 73% of the total variance accounted for by PC1 and 27% by PC2. This shows that the separation between the samples is strong and reliable. PCA results of C-YCJ, P-YCJ and TS-YCJ samples are given in Figure 9.

When the clusters of the samples are examined, the C-YCJ samples are located in the negative PC1 region on the left side of the plot. They are associated with free amino acids (glycine, phenylalanine, histidine, proline, and ornithine). This shows that the control samples have high amino acid content, and these components may be preserved in the control group. Proline, alanine, and histidine are notable components of C-YCJ. Additionally, the control group is located in the negative PC2 region and is associated with low values in terms of sensory and functional properties.

Pasteurized samples (P-YCJ) are located in the negative PC1 and negative PC2 regions in the lower left part of the graph. This situation illustrates the negative effects of pasteurization, particularly on malic acid and phenolic compounds. Malic acid and TPC content decreased after pasteurization. For example, while malic acid content is at the highest level in the thermosonication sample, this level is lower in the pasteurized sample, indicating the loss of compounds due to heat treatment.

Thermosonicated samples (TS-YCJ) are concentrated in the positive PC1 and positive PC2 regions on the right side of the graph. This situation reveals that the application of thermosonication has a strong, positive correlation with bioactive and sensory parameters, including DPPH, chlorogenic acid, caffeic acid, catechin, epicatechin, and total soluble solids (TSS). For example, antioxidant capacity (DPPH) reached the highest level in thermosonication application, and these properties are represented by long vectors on the positive axis. This demonstrates that thermosonication effectively preserves bioactive compounds and enhances their functional properties.

These clusters show how the process conditions (control, pasteurization, and thermosonication) affect bioactive and sensory parameters in the PCA plot. The parameters concentrated in the positive PC1 and PC2 regions indicate that thermosonication enhances functional and sensory quality; the parameters remaining in the negative region suggest that pasteurization may negatively impact bioactive components. Additionally, the association of free amino acids in the negative PC1 region of C-YCJ samples indicates that the control group has a more natural and unprocessed component profile. These results demonstrate that thermosonication has a positive impact on both functional and sensory properties.

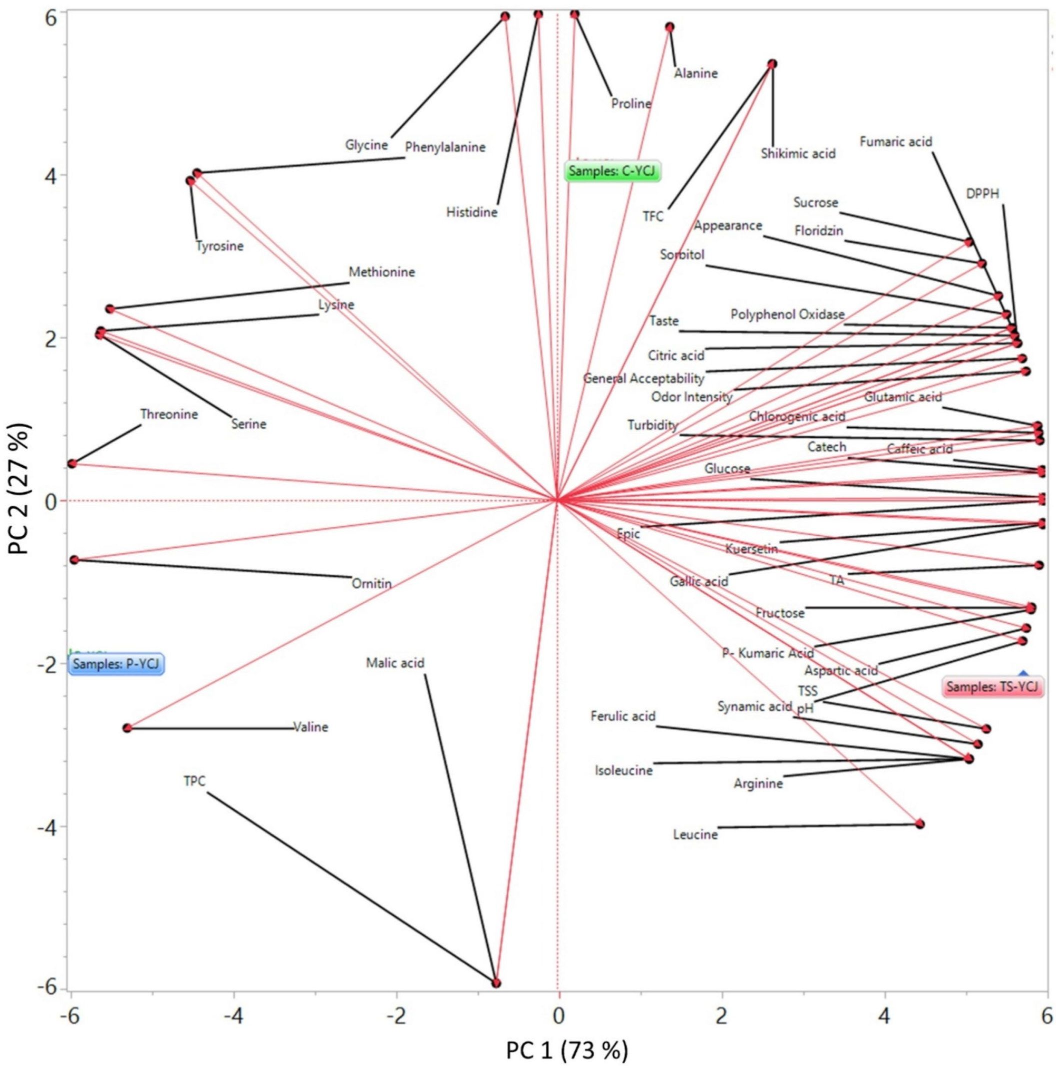

FIGURE 9
PCA result of C-YCJ, P-YCJ, and TS-YCJ samples.

4 Conclusion

This study successfully demonstrated the effects of propolis enrichment and thermosonication on the bioactive components, amino acid profile, antioxidant capacity, and sensory properties of yellow cherry juice. Thermosonication application resulted in a significant increase in total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity, as measured by DPPH inhibition. The malic acid content reached its highest level of 1,174.38 mg/L in thermosonicated samples (TS-YCJ), while it remained at lower levels in the control (C-YCJ) and pasteurized (P-YCJ) samples. Chlorogenic acid (35.42 mg/L) and caffeic acid (12.67 mg/L) from phenolic compounds increased significantly after thermosonication. In terms of amino acid profile, proline, glycine, and phenylalanine were found at higher levels in control samples. Sensory analysis results showed that thermosonicated samples had higher scores in terms of taste, odor, and general acceptability. These results suggest that a combination of thermosonication and propolis provides an effective approach to improve the functional and sensory properties of yellow cherry juice. Future studies should be supported by studies on cell culture and laboratory animals to determine the bioavailability and health effects of these results.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

Author contributions

SY: Supervision, Investigation, Methodology, Conceptualization, Data curation, Validation, Writing – review & editing, Writing – original draft, Project administration. MT: Writing – review & editing, Formal analysis, Validation, Writing – original draft, Conceptualization. NTD: Software, Writing – review & editing, Validation, Writing – original draft, Visualization. NT: Resources, Writing – original draft, Writing – review & editing, Formal analysis. ER: Formal analysis, Writing – original draft, Writing – review & editing. IM: Writing – review & editing, Writing – original draft. MOA: Funding acquisition, Writing – review & editing, Writing – original draft.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. Princess Nourah bint Abdulrahman University Researchers Supporting Project Number (PNURSP2025R251), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Acknowledgments

The authors are grateful for the support from Princess Nourah bint Abdulrahman University Researchers Supporting Project Number (PNURSP2025R251), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia, for their generous support in this research. The authors gratefully acknowledge the financial support provided by TÜBİTAK (Scientific and Technological Research Council of Turkey) under the 2209-A Student Research Project Program (project number 1919B012321210).

Conflict of interest

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