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Advancing sustainable juice processing through thermosonication: functional enrichment of apple juice with pollen

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The development of functional beverages requires the use of sustainable technologies to increase both nutritional value and consumer appreciation. This study aims to investigate the effects of thermosonication, a sustainable food processing approach, on Uruset apple juice to produce a functional beverage enriched with pollen. In the study, four process parameters—temperature, time, amplitude, and pollen concentration—were optimized. As a result of the experimental design using response surface methodology (RSM), significant increases were observed in total phenolic substance (TPC), total flavonoid (TFC), and antioxidant capacity [DPPH (2,2-diphenyl-1-picrylhydrazyl) and CUPRAC (copper reducing antioxidant capacity)] values. At the same time, significant improvements were observed in the content of phenolic compounds, including chlorogenic acid, gallic acid, quercetin, and catechin. Thermosonication process partially suppressed the enzymatic activity (PPO) and ensured the preservation of phenolic compounds and positively affected the shelf life of the product. Sensory analysis results showed that apple pollen juice processed with thermosonication received higher scores in terms of taste, odor, and general liking compared to both the control and pasteurized groups. Optimum process conditions were determined to be 40.9°C temperature, 5.63 min process time, 61.01% amplitude, and 60 mg/100 mL pollen. The findings reveal that a sustainable, functional beverage with enhanced nutritional and sensory quality can be developed through the synergistic effect of thermosonication and a natural pollen additive. This approach offers an innovative and environmentally friendly solution that can be applied on an industrial scale as an alternative to traditional heat treatments. In the future, the production of functional beverages supported by innovative processes such as thermosonication could help to promote healthier and more environmentally friendly products within the food industry.

KEYWORDS

sustainable food processing, apple juice, functional beverage, pollen, thermosonication, phenolic compounds

1 Introduction

Fruit juices are important sources of bioactive compounds such as vitamin C, carotenoids, and phenolics. The world's most consumed fruit juice, particularly in Europe and North America, is apple (Franz et al., 2009; Lu et al., 2010). Uruset apple juice (red inside and out) contains more bioactive compounds than other apple juices (Yıkmış, 2019; Coşkun and Aşkın, 2016; Abacı and Sevindik, 2014). Ultrasonication is a term defined as "the generation of energy by sound waves that vibrate at 20,000 or more vibrations per second" (Lima et al., 2015; Zhang et al., 2016). Ultrasonic equipment generally operates at frequencies between 20 kHz and 10 MHz. Fruit juices are susceptible to deterioration from heat, micro-organisms, enzymes, oxygen and light during processing and storage (Liang et al., 2006). Compared to heat treatment, ultrasonication inactivates pathogenic microorganisms and enzymes that cause spoilage. In addition, the low temperature applied in the process helps preserve the product's taste, smell, texture, and nutrients. The result is a product with characteristics very close to its fresh form (Tiwari and Mason, 2012; McClements, 1995; Leighton, 2007; Chandrapala et al., 2012). The effect of the ultrasound process can be increased by using temperature, pressure, or both. The combination of ultrasound with moderate heat is referred to as thermosonication; the combination with pressure is termed manosonication; the use of ultrasound in conjunction with ultraviolet light is designated photosonication; and the combination of ultrasound with both temperature and pressure is referred to as manothermosonication (Chemat et al., 2011; Şengül et al., 2011). Pollen, a natural ingredient, was added in this study to increase the nutritional content and functional properties of apple juice. Bee pollen is a mixture of flower pollen from various botanical sources, collected by bees and agglutinated by enzymes and nectar, and is a valuable product of the beehive (Bertoncelj et al., 2023). Bee pollen is a good food for bees and people. It consists of 13–55% carbohydrates, 10–40% protein, 0.2–3.0% mineral substances, 1–12% lipids, 0.3–20% fibre, and other organic compounds (İzol et al., 2025). The flavonoids found in bee pollen include kaempferol, quercetin, delphinidin, luteolin, naringenin, galangin, isorhamnetin, rutin, pinocembrin, catechin and apigenin (Yildiz and Maskan, 2022).

In a study investigating the effect of thermosonication treatment (20 kHz; 30 μm; 5 min; 30°C) on bioactive compounds of watermelon (*Citrullus lanatus* cv.) juice, it was found that the coefficient of determination (R^2) for the estimated quality parameters showed good correlation with the experimental data at a confidence level of 95% (Rawson et al., 2011). In another study, the application of thermosonication (15 and 25 min, 80% amplitude) on the shelf life and the antioxidant, microbiological, and physicochemical properties of prickly pear (*Opuntia ficus-indica*) juice was investigated. It was reported that after 14 days of storage, especially for 80% and 25 min treatment, the phenolic content increased, and there was an increase in antioxidant activity [2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and DPPH] until the end of storage (Del Socorro Cruz-Cansino et al., 2015). Carrot juice was sonicated at 50°C, 54°C, and 58°C for 10 min (24 kHz, 120 μm amplitude) (acoustic power 2204.40, 2155.72, 2181.68 mW/mL, respectively). The researchers evaluated the thermosensation process as a promising technology to minimize physicochemical changes during storage, delay microbial growth, and improve the quality of carrot juice by preserving bioactive compounds (Martínez-Flores et al., 2015). Thermosonication is a new and applicable technique used instead of traditional heat treatments. It has been reported that ultrasonication is generally more effective when combined with moderate heat. It has been stated that this combined process increases microbial and enzymatic inactivation with heat and cavitation; this is achieved without altering the quality of the juice (Anaya-Esparza et al., 2017).

After the literature review, Uruset apple juice was preferred because of the high bioavailability of Uruset apple juice and the fact that there is no study on thermosonication on Uruset apple juice. This study aims to develop Uruset apple juice as a functional beverage with the addition of pollen. Concurrently, response surface methodology was employed to optimize the beverage's bioactive components and antioxidant properties. Furthermore, a comprehensive comparative analysis was conducted between pollen-fortified thermosonicated apple juice and both thermally pasteurized and untreated control samples. The evaluated parameters included: bioactive characteristics [phenolic compound composition and polyphenol oxidase (PPO) activity], physicochemical properties (pH, titratable acidity and soluble solids content), biochemical composition (sugar content and organic acid profile), as well as sensory attributes.

2 Materials and methods

2.1 Material

The research used Uruset apple grown in the Ardahan region as material. Analyses were conducted in Tekirdağ Namık Kemal University Çorlu Vocational School Food Technology Laboratories.

2.2 Preparation of Uruset apple juice concentrate and pollen addition

Uruset apple juice was first made by breaking fresh Uruset apple fruits in a blender (ISO LAB Blender, 602.21.001) at low speed for 30 s and obtaining fruit pulp. The mixture was filtered. The prepared concentrates were stored in 50 mL sterile sample containers with lids at −18°C. Pollen samples were obtained from different beekeepers operating in Tekirdağ and were used in the analyses after being mixed and homogenized.

2.3 Experimental design of response surface methodology

Uruset apple juice was analyzed using the response surface method Minitab Statistical Analysis Software (Minitab 18.1.1) to understand the effect of thermosonication application on quality parameters. Response surface method (RSM) was used. A five-level, three-factor central composite design was chosen as the experimental design. There are 31 trial points for optimization (Tables 1, 2). The adequacy of the model was assessed by

TABLE 1 Independent variable values and their corresponding proportions used in response surface method.

Independent variables	Level				
	−1.68	−1	0	1	1.68
X_1: Temperature (°C)	30	35	40	45	50
X_2: Time (min.)	2	4	6	8	10
X_3: Amplitude (%)	40	50	60	70	80
X_4: Pollen (mg/100 mL)	40	50	60	70	80

considering the R^2 and the adjusted -R^2 coefficients, the lack of fit tests, and the ANOVA results. Independent variables were determined as temperature (X_1), time (X_2), amplitude (X_3), and pollen (X_4). Dependent variables were selected, such as phenolic substance, flavonoid substance, and antioxidant values. The second-degree-polynomial equation shown in the following equation was used to create the model Equation 1:

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

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, β_{ij} is the two-factor cross-interaction coefficient, and X_i and X_j are the independent variables.

2.4 Determination of bioactive compounds

The total phenolic content amount was determined spectrophotometrically using the Folin–Ciocalteu method with modifications. The absorbance was measured with a UV–VIS spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Victoria, Australia) at a wavelength of 765 nm. Subsequently, the findings were expressed as milligrams of gallic acid equivalents per liter (mg GAE/L) (Zhishen et al., 1999). Total flavonoid content was determined by the aluminum chloride colorimetric analysis method. The total content of flavonoids is expressed in mg of quercetin equivalents (CE/L) (Singleton et al., 1999). The CUPRAC (Cu (II) ion-reducing antioxidant capacity) method has been described in a previous study by Apak et al. (2006), and the DPPH scavenging activity method, as described by Singh et al. (2002), was used to determine the antioxidant capacity of Uruset apple juice. Analyses were performed in triplicate.

2.5 Physicochemical analysis

The temperature of the samples to be measured for pH was brought to +20°C, and the pH analysis of the samples was performed using the Hanna Instruments HI 2002 pH/ORP device (Cemeroğlu, 2010). Analyses were performed in triplicate. The refractometric method determined the amount of dissolved dry matter (Cemeroğlu, 2010). Measurements were made at 20°C using a PCE-032 brand model refractometer, and the results were expressed as °Brix. Analyses were performed in triplicate. Titratable acidity was determined potentiometrically by titrating the samples with 0.1 N NaOH solution to pH 8.1 (Sadler and Murphy, 2010). Analyses were performed in triplicate.

2.6 Analysis of organic acid and sugar

The amount of organic acids in the samples was determined according to the method specified by Castellari et al. (2000). The method was established by preparing standards in the expected concentration ranges for organic acids (citric, malic, ascorbic) and sugars (sucrose, glucose, fructose). Citric and malic acids from organic acids were determined at 210 nm and ascorbic acid at 243 nm with the diode array detector (DAD detector). Results are given as mg/100 mL for organic acid. The amount of sugar in the samples was determined according to the method specified by Sturm et al. (2003). Sugars were determined in the refractive index detector (RID detector). Sugar results are given in g/100 mL.

2.7 Analysis of phenolic compounds

Phenolic compounds were analyzed using an Agilent 1,260 Infinity chromatograph with a diode array detector (DAD). As outlined in the study by Portu et al. (2017), the chromatography process was carried out utilizing a C-18 Agilent column (250 × 4.6 mm; 5 µm packing). The column temperature was fixed at 30°C with a flow rate of 0.80 mL/min. Detection was carried out at 280, 320, and 360 nm. The concentrations of these compounds are expressed as µg/mL. The results for phenolic compounds are given as the average of the analyses of three samples.

2.8 Analysis of polyphenol oxidase enzyme (PPO) activity

0.1 mL of Uruset apple juice and 2.8 mL (0.1 M, pH = 6.8) Na-phosphate buffer solution was added to the cuvette in the spectrophotometer cuvette holder. The solution was left for 3 min to extract PPO. At the end of the incubation period, 0.1 mL (0.1 M) catechol solution was added and mixed, and absorbance measurement was started immediately 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. Using the slope of the graph, PPO enzyme activity was calculated as Unit/mL (U/mL) as specified in Cemeroğlu (2010).

10.3389/fsufs.2025.1601419

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) (%)	Pollen (mg/100 mL) (X_4)	TPC (mg GAE/L)		TFC (mg CE/L)		DPPH (inhibition %)		CUPRAC (inhibition %)	
					Experimental data	RSM predicted	Experimental data	RSM predicted	Experimental data	RSM predicted	Experimental data	RSM predicted
1	35	4	70	70	861.44	860.77	344.58	344.63	55.40	55.65	59.72	59.98
2	35	4	70	50	856.58	857.16	342.63	342.98	58.07	58.14	60.11	60.03
3	45	4	70	70	874.72	875.42	349.89	349.97	59.30	59.44	62.37	62.15
4	40	6	60	60	870.56	870.42	355.62	355.51	60.27	60.27	64.02	64.24
5	40	6	60	60	870.56	870.42	355.62	355.51	60.27	60.27	64.14	64.24
6	40	6	60	60	870.48	870.42	355.34	355.51	60.23	60.27	64.37	64.24
7	30	6	60	60	862.16	862.34	346.75	346.08	59.27	59.08	61.81	61.55
8	40	6	40	60	864.07	863.66	346.65	346.03	59.38	59.19	61.44	61.07
9	40	6	60	60	870.56	870.42	355.62	355.51	60.27	60.27	64.38	64.24
10	35	4	50	50	867.57	867.40	347.03	347.45	59.61	59.91	60.87	60.98
11	45	8	50	70	853.78	853.95	341.51	341.55	57.88	57.95	59.91	59.80
12	40	10	60	60	859.27	859.70	343.71	343.60	57.27	56.99	60.73	60.98
13	40	6	80	60	865.86	866.35	346.34	346.60	58.7	58.42	60.18	60.51
14	45	8	50	50	860.12	860.30	344.05	344.13	54.31	54.52	60.36	60.23
15	50	6	60	60	868.84	868.73	347.54	347.84	58.90	58.62	60.4	60.63
16	45	8	70	50	867.54	867.53	347.02	347.15	56.33	56.59	60.88	60.52
17	40	6	60	60	870.12	870,42	355.24	355.51	60.21	60.27	64.32	64.24
18	40	6	60	40	865.13	865.22	346.05	345.80	57.66	57.36	60.71	60.84
19	45	4	50	50	866.72	867.26	346.69	346.87	58.76	58.85	59.73	59.88
20	45	8	70	70	867.21	866.88	346.88	346.60	58.79	58.95	60.16	60.19
21	40	2	60	60	867.43	867.07	346.97	346.72	58.81	58.61	60.87	60.60
22	40	6	60	60	870.12	870.42	355.62	355.51	60.27	60.27	64.39	64.24
23	35	8	70	50	863.66	863.22	345.46	345.44	58.55	58.78	61.61	61.61
24	35	8	50	70	860.12	860.00	344.05	344.49	59.10	59.41	61.36	61.62
25	35	4	50	70	864.55	865.31	346.82	347.07	58.61	58.49	60.67	60.83
26	35	8	70	70	860.14	860.35	344.06	344.26	58.31	58.36	60.35	60.01
27	45	4	50	70	867.43	867.38	346.97	347.13	59.98	60.21	60.87	61.00
28	35	8	50	50	868.51	868.57	347.40	347.70	58.77	58.77	63.31	63.33
29	40	6	60	80	862.49	862.48	345.00	344.88	58.47	58.30	60.53	60.36

(Continued)

TABLE 2 (Continued)

Run no.	Independent variables				Dependent variables							
	Temperature (X$_1$) (°C)	Time (X$_2$) (min.)	Amplitude (X$_3$) (%)	Pollen (mg/100 mL) (X$_4$)	TPC (mg GAE/L)		TFC (mg CE/L)		DPPH (inhibition %)		CUPRAC (inhibition %)	
					Experimental data	RSM predicted	Experimental data	RSM predicted	Experimental data	RSM predicted	Experimental data	RSM predicted
30	40	6	60	60	870.12	870.42	355.34	355.51	60.23	60.27	64.14	64.24
31	45	4	70	50	869.97	869.60	347.99	347.68	58.98	59.13	61.05	60.93
TS-UJ	40.90	3.63	61.01	60.00	871.04		355.58		60.27		64.14	
Experimental values					682.63		441.87		61.80		67.51	
% Difference					27%		%0.24		%0.02		%4.9	

X$_1$: temperature; X$_2$: time; X$_3$: amplitude; X$_4$: Pollen; TS-UJ: Thermosonicated optimized pollen Uruset apple juice; TPC: total phenolic content; TFC: total flavonoid content; DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant capacity; CUPRAC: copper reducing antioxidant capacity; GAE: gallic acid equivalent; CE: quercetin equivalents.

2.9 Sensory analysis

Ultrasound-treated pollen-fortified Uruset apple juice was compared with thermal pasteurized apple juice and fresh Uruset apple juice. The study was conducted in two replicates. Panelists were asked to evaluate the samples' taste, odor, color, texture, and general acceptability. Fifty trained panelists participated in the sensory evaluation of the juice samples. All samples were coded using a random three-digit alphabet. Sensory properties were determined using a 9-point hedonic scale (0–9). Scale scores: excellent, 9; very good, 8; good, 7; acceptable, 6; poor (initial odorless, tasteless development) < 6; lower score accepted 6. The product was considered unacceptable after the first odor or tastelessness (Portu et al., 2017).

2.10 Statistical analysis

Statistical analyses of the comparative analyses of the study were performed using the SPSS 20.0 (SPSS Inc., Chicago, United States) program. Samples were compared using the 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

This study evaluated the effects of temperature (X_1), time (X_2), amplitude (X_3) and pollen concentration (X_4) on the bioactive properties of Uruset apple juice using RSM. Dependent and independent variables are shown in Table 2. The optimization process was carried out to increase total phenolic substance (TPC), total flavonoid substance (TFC) and antioxidant activities (DPPH and CUPRAC methods). The effects of temperature (X_1), time (X_2), amplitude (X_3) and pollen (X_4) independent variables on TPC (Equation 2), TFC (Equation 3), DPPH (Equation 4) and CUPRAC (Equation 5) properties of Uruset apple juice are shown in the following equations. ANOVA results are given in Table 3.

$$\begin{aligned} TPC\left(mg\frac{GAE}{L}\right) = {} & 834.6 + 1.001\,X_1 + 13.651\,X_2 - 2.047\,X_3 \\ & + 1.089\,X_4 - 0.04878\,X_1X_1 - 0.4393\,X_2X_2 \\ & - 0.013533\,X_3X_3 - 0.016421\,X_4X_4 - 0.2030\,X_1X_2 \\ & + 0.06290\,X_1X_3 + 0.01108\,X_1X_4 + 0.06119\,X_2X_3 \\ & - 0.08087\,X_2X_4 + 0.01425\,X_3X_4 \end{aligned} \quad (2)$$

$$\begin{aligned} TFC\left(mg\frac{CE}{L}\right) = {} & 88.9 + 5.603\,X_1 + 10.846\,X_2 + 1.247\,X_3 \\ & + 2.809\,X_4 - 0.08551\,X_1X_1 - 0.6471\,X_2X_2 \\ & - 0.022992\,X_3X_3 - 0.025424\,X_4X_4 - 0.07495\,X_1X_2 \\ & + 0.02641\,X_1X_3 + 0.00318\,X_1X_4 + 0.02760\,X_2X_3 \\ & - 0.03547\,X_2X_4 + 0.005075\,X_3X_4 \end{aligned} \quad (3)$$

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

		TPC (mg GAE/L)		TFC (mg CE/L)		DPPH (inhibition %)		CUPRAC (inhibition %)	
Source	DF	F-value	*P*-value	F-value	*P*-value	F-value	*P*-value	F-value	*P*-value
Model	14.00	210.82	0.000	290.18	0.000	66.17	0.000	72.64	0.000
Linear	4.00	175.77	0.000	37.00	0.000	25.15	0.000	7.39	0.001
X_1	1.00	260.67	0.000	32.66	0.000	5.14	0.038	16.26	0.001
X_2	1.00	347.84	0.000	102.68	0.000	61.24	0.000	2.78	0.115
X_3	1.00	45.77	0.000	3.34	0.086	13.85	0.002	6.07	0.025
X_4	1.00	48.78	0.000	9.30	0.008	20.36	0.000	4.44	0.051
Square	4.00	212.66	0.000	882.76	0.000	87.62	0.000	205.95	0.000
X_1X_1	1.00	181.59	0.000	922.09	0.000	55.91	0.000	229.37	0.000
X_2X_2	1.00	376.91	0.000	1351.95	0.000	168.72	0.000	275.81	0.000
X_3X_3	1.00	223.60	0.000	1066.68	0.000	59.59	0.000	274.39	0.000
X_4X_4	1.00	329.19	0.000	1304.33	0.000	164.64	0.000	305.83	0.000
2-way interaction	6.00	232.97	0.000	63.91	0.000	79.22	0.000	27.26	0.000
X_1X_2	1.00	281.49	0.000	63.42	0.000	157.11	0.000	51.47	0.000
X_1X_3	1.00	675.64	0.000	196.87	0.000	65.39	0.000	51.86	0.000
X_1X_4	1.00	20.95	0.000	2.85	0.111	120.32	0.000	21.00	0.000
X_2X_3	1.00	102.30	0.000	34.40	0.000	49.18	0.000	7.53	0.014
X_2X_4	1.00	178.72	0.000	56.83	0.000	65.87	0.000	31.54	0.000
X_3X_4	1.00	138.71	0.000	29.08	0.000	17.45	0.001	0.14	0.718
Error	16.00								
Lack-of-fit	10.00	6.72	0.015	7.18	0.013	122.76	0.000	4.98	0.031
Pure error	6								
Total	30								
R^2		99.46%		99.61%		98.30%		98.45%	
Adjusted R^2		98.99%		99.26%		96.82%		97.10%	
Predicted R^2		97.09%		97.87%		90.26%		91.81%	

X_1: temperature; X_2: time; X_3: amplitude; X_4: Pollen; DF—degrees of freedom; R^2—coefficient of determination. TPC: total phenolic substance; TFC: total flavonoid, antioxidant capacity; DPPH: 2,2-diphenyl-1-picrylhydrazyl; CUPRAC: copper reducing antioxidant capacity. $p < 0.05$, statistically significant; $p < 0.01$, statistically highly significant.

$$\begin{aligned}\text{DPPH}\left(\text{inhibition\%}\right) = {} & 45.45 + 0.139\,X_1 + 1.951\,X_2 \\ & + 0.0355\,X_3 + 0.2022\,X_4 - 0.01422\,X_1X_1 \\ & - 0.1543\,X_2X_2 - 0.003669\,X_3X_3 - 0.006098\,X_4X_4 \\ & - 0.07964\,X_1X_2 + 0.01028\,X_1X_3 + 0.01394\,X_1X_4 \\ & + 0.02228\,X_2X_3 + 0.02578\,X_2X_4 - 0.002654\,X_3X_4 \end{aligned} \quad (4)$$

$$\begin{aligned}\text{CUPRAC}\left(\text{inhibition\%}\right) = {} & -36.63 + 1.788\,X_1 + 6.369\,X_2 \\ & + 0.6602\,X_3 + 0.9251\,X_4 - 0.03146\,X_1X_1 \\ & - 0.2156\,X_2X_2 - 0.008603\,X_3X_3 - 0.009083\,X_4X_4 \\ & - 0.04981\,X_1X_2 + 0.01000\,X_1X_3 + 0.00636\,X_1X_4 \\ & - 0.00953\,X_2X_3 - 0.01950\,X_2X_4 + 0.000256\,X_3X_4 \end{aligned} \quad (5)$$

ANOVA results showed that all independent variables (X_1–X_4) had a significant effect on the measured responses ($p < 0.05$). Temperature and time were found to have the most significant effects.

3.1.1 Total phenolic compound (TPC)

All factors showed high significance ($p < 0.0001$) in second-order effects. The highest F-values were recorded for X_2 ($F = 347.84$) and X_4 ($F = 329.19$), indicating that the duration and pollen amount were important in preserving phenolic compounds (Figure 1). Especially the increase in duration had a positive effect on total phenolic compounds, and this result is consistent with previous studies on the enrichment of phenolic compounds (Gadioli Tarone et al., 2021; Kaur et al., 2022).

Total flavonoid substance (TFC), especially X_2 ($F = 1351.95$) and X_4 ($F = 1304.33$) variables, have the highest effect, revealing that time and pollen concentration play a critical role in the preservation of these compounds. An increase in the amount of TFC was observed in thermosonication treatment (Figure 2). Similar results were observed in samples of orange juice, whey (Oliveira et al., 2022), black, red, and white currant juices (Kidoń and Narasimhan, 2022).

Figures 3, 4 show response surface plots of DPPH and CUPRAC, respectively, RSM as a function of the significant interaction factors.

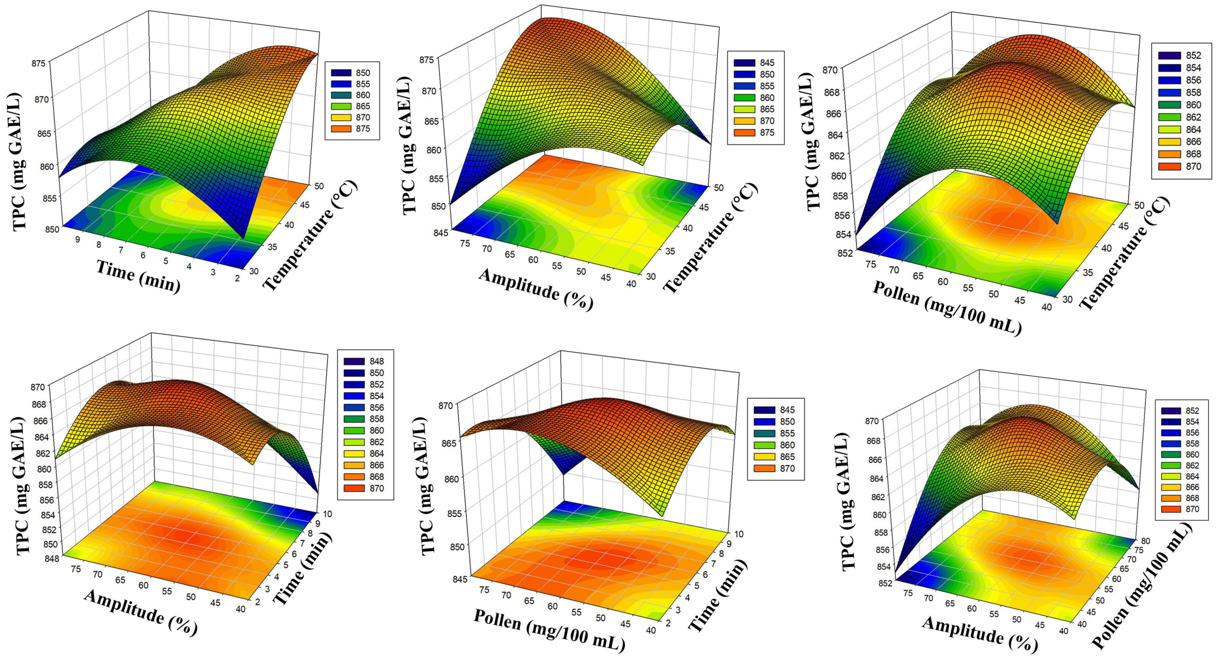

FIGURE 1
Response surface plots (3D) of TPC RSM as functions of significant interaction factors.

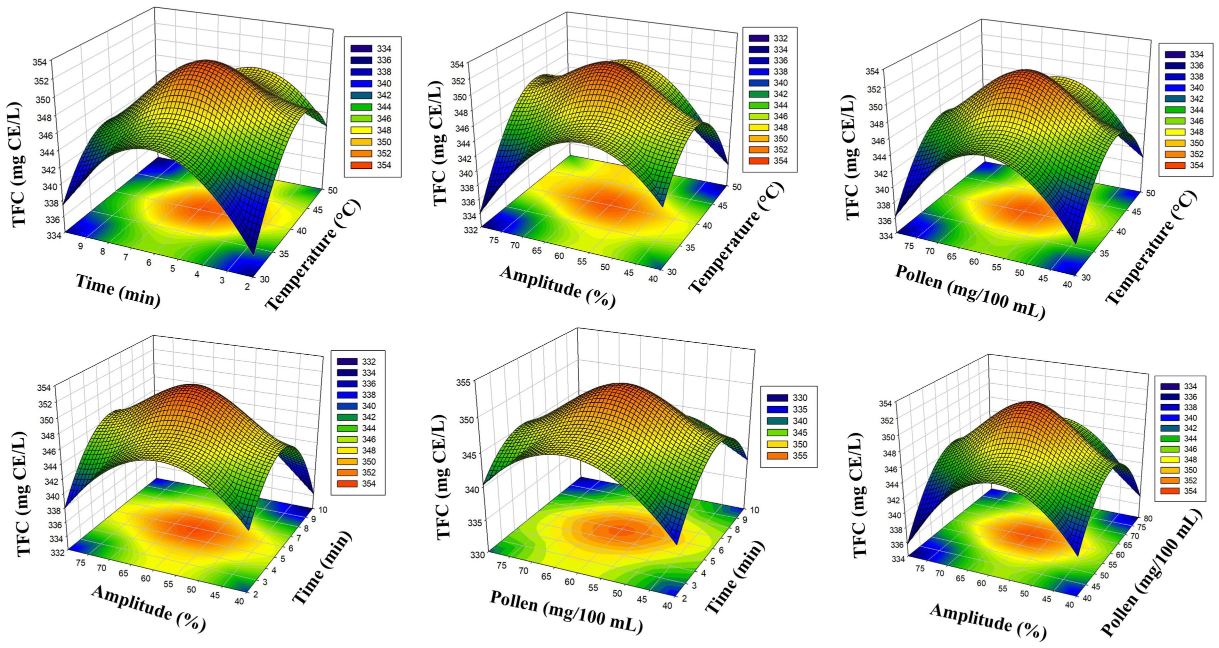

FIGURE 2
Response surface plots (3D) of TFC RSM as functions of significant interaction factors.

Significant improvements were observed with increasing temperature and amplitude in both methods, confirming the potential of ultrasound-assisted processing to enhance the extraction of bioactive compounds.

Increasing thermosonication parameters, as in our study, was reported to increase antioxidant activity in black carrot juice (Hasheminya and Dehghannya, 2022). Similar results were observed in other studies where thermosonication application to cashew apple juice and amora (*Spondius pinnata*) juice increased antioxidant activity values (Deli et al., 2022; Nayak et al., 2022). Pairwise interactions revealed complex relationships between variables and were statistically significant ($p < 0.01$). X_1 and X_2

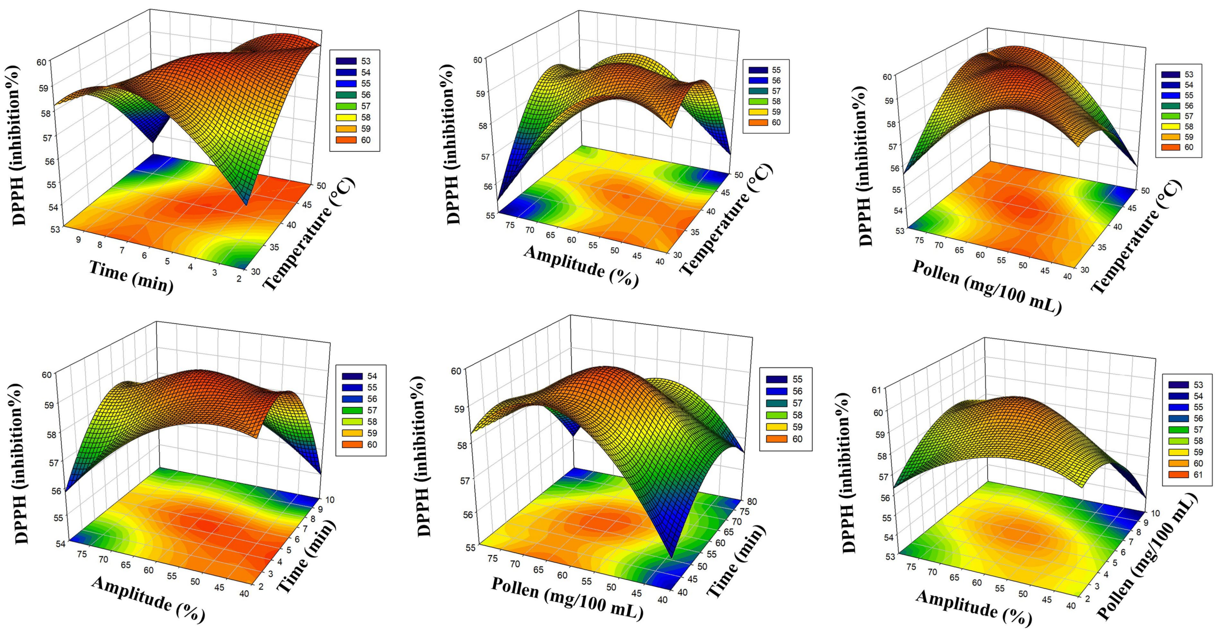

FIGURE 3
Response surface plots (3D) of DPPH RSM as functions of significant interaction factors.

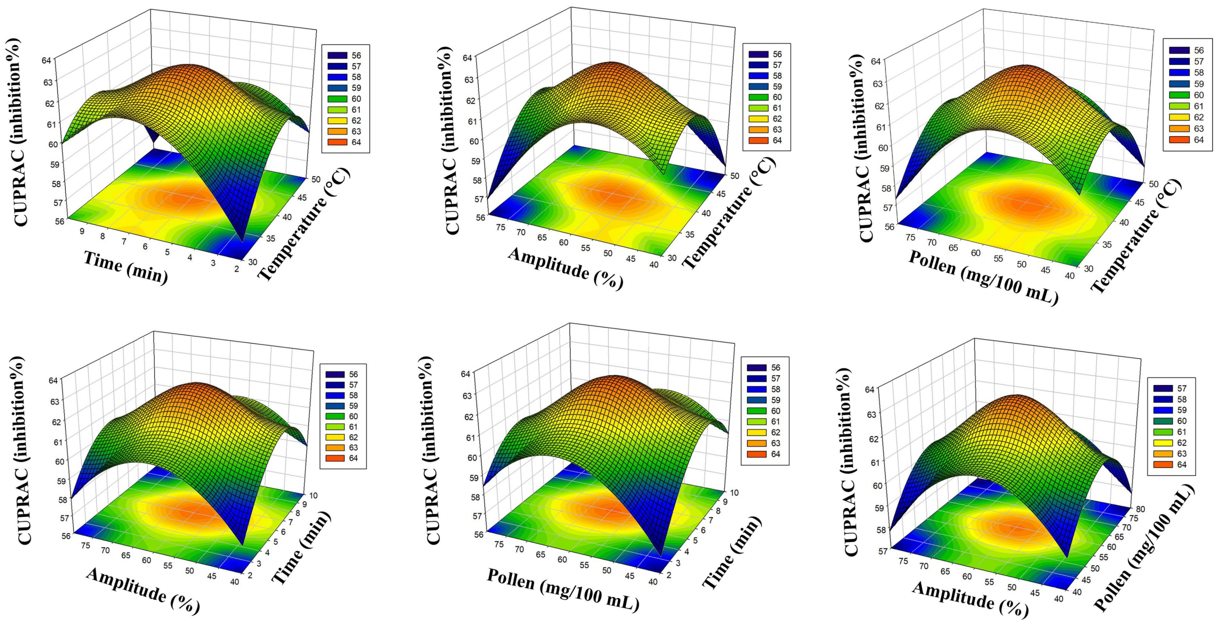

FIGURE 4
Response surface plots (3D) of CUPRAC RSM as functions of significant interaction factors.

were found to significantly affect all responses, particularly a strong synergy with the CUPRAC value ($F = 51.47$). X_1 and X_3 were found to be significant, especially for TPC ($F = 675.64$) and TFC ($F = 196.87$), indicating the effect of ultrasound treatment in enhancing the extraction of phenolic compounds. $X_2 \times X_4$ had high significance on DPPH ($F = 65.87$) and CUPRAC ($F = 31.54$), indicating that longer treatment time could increase antioxidant capacity by increasing pollen content.

The coefficient of determination (R^2) values are above 98%, indicating excellent agreement between experimental and predicted data. The adjusted R^2 and predicted R^2 values are also exceptionally high, proving the reliability of the developed models. These findings show that the predictive power of RSM models is relatively high and can be used safely in optimization processes. The optimum process conditions were determined as 40.9°C temperature, 5.63 min process time, 61.01% amplitude, and 60 mg/100 mL pollen. The difference

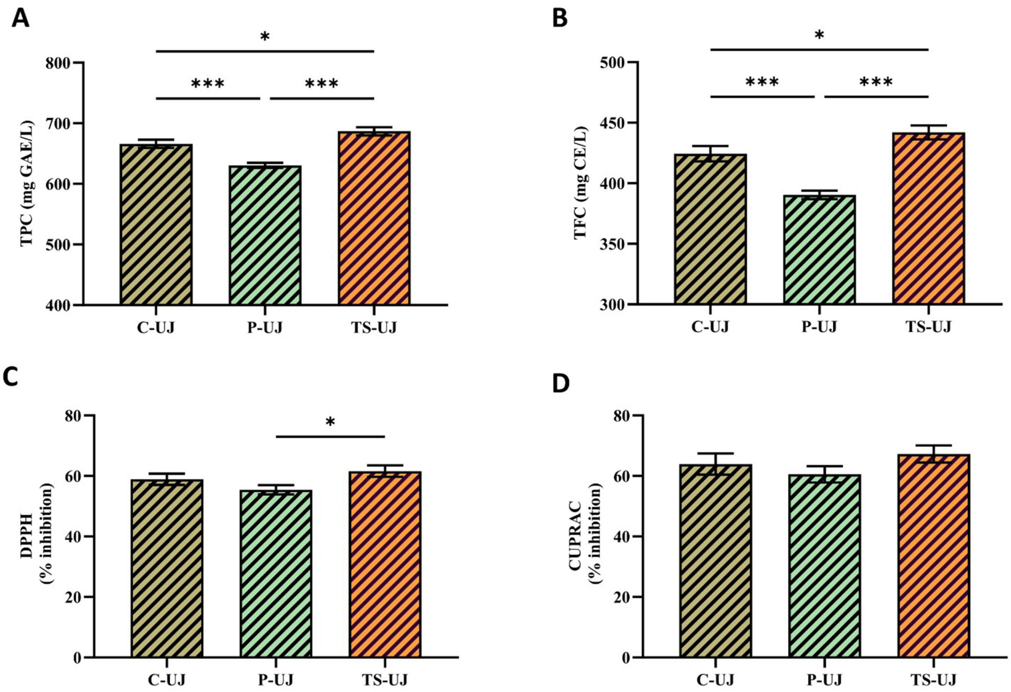

FIGURE 5
Results for the total phenolic compounds **(A)**, total flavonoid compounds **(B)**, DPPH: 2,2-diphenyl-1-picrylhydrazyl **(C)**, and CUPRAC: copper reducing antioxidant capacity **(D)** of C-UJ: Control Uruset apple juice; P-UJ: Pasteurized Uruset apple juice; TS-UJ: Thermosonicated optimized pollen Uruset apple juice.

between experimental and predicted values is less than 5%, which supports the reliability of the optimization process. This study provides a scientific basis for the industrial-scale development of Uruset apple juice and offers important findings for the production of functional beverages. In particular, the potential of ultrasound-assisted processing to increase the bioavailability of bioactive compounds is of great importance for developing functional products for consumer health.

3.2 Bioactive compounds

Fruits are the preferred source of bioactive compounds with health benefits for preventing and/or treating disease (De La Fuente-Carmelino et al., 2025). The main bioactive compounds are secondary metabolites with antioxidant, antimicrobial, anti-inflammatory, and other properties (Meena et al., 2024). Figure 5 shows significant differences ($p < 0.05$) in total phenolic compounds, flavonoid compounds, DPPH, and CUPRAC content. TPC and TFC were significantly affected by thermosonication, with the highest values observed in these samples. The results are parallel to previous studies that found that the thermosonication process increased TPC and TFC values (Yıkmış et al., 2022; Giovagnoli-Vicuña et al., 2022). The present study observed a significant difference in DPPH values between P-UJ and TS-UJ samples. Similarly, Mukhtar et al. (2024) found that the applied thermosonication process (25 kHz at 40 and 50°C) provided a significant increase in DPPH value compared to heat treatment (100°C, 4 min) of watermelon-beetroot juice. Additionally, Li et al. (2025) reported that thermosonication process (60°C, 15 min) provided significantly higher antioxidant activity compared to the pasteurization process (85°C for 10 min).

Pearson correlation plot shows the effect of thermosonication and pollen addition on physicochemical, sensory and bioactive properties in Uruset apple juice. A very strong positive correlation ($r > 0.99$) was observed between sugar components (fructose, glucose, sucrose and sorbitol), indicating that the components increased or decreased together. Also, a high level of positive correlation ($r > 0.95$) was found between total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity (DPPH and CUPRAC), indicating that thermosonication and pollen addition increased the preservation and extraction of bioactive compounds (Figure 6).

3.3 Physicochemical parameters

In terms of physicochemical properties, pH levels remain relatively constant between treatments and are slightly higher than TS-UJ (3.57), C-UJ (3.54), and P-UJ (3.53) (Figure 7). Similar to these results, no significant change in pH value was observed after thermosonication treatment in the hog plum juice study conducted by Oladunjoye et al. in 2021 (Oladunjoye et al., 2021). The finding that pH value from physicochemical parameters did not show any significant difference compared to the control group is consistent with the result of this study. Total soluble solids (TSS, °Brix) content was the highest in TS-UJ (14.14), followed by C-UJ (13.80) and P-UJ (13.77), indicating that thermosonication may contribute to the release of soluble compounds and increase the sweetness and flavor of the juice. Titratable acidity (TA) remained relatively unchanged, with

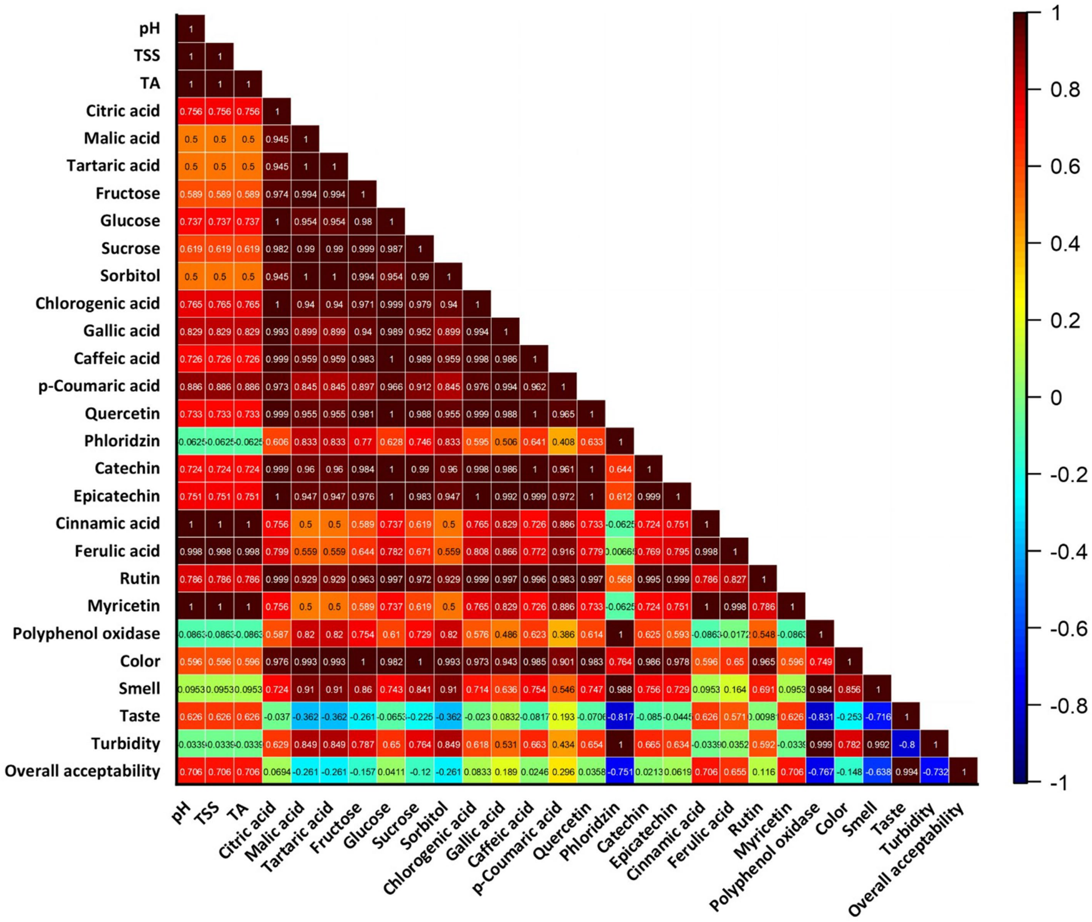

FIGURE 6
Pearson correlates the coefficients between the physicochemical parameters, organic acid, sugar, phenolic compounds, polyphenol oxidase enzyme activity, sensory properties of the optimized thermosonicated Uruset apple juice samples.

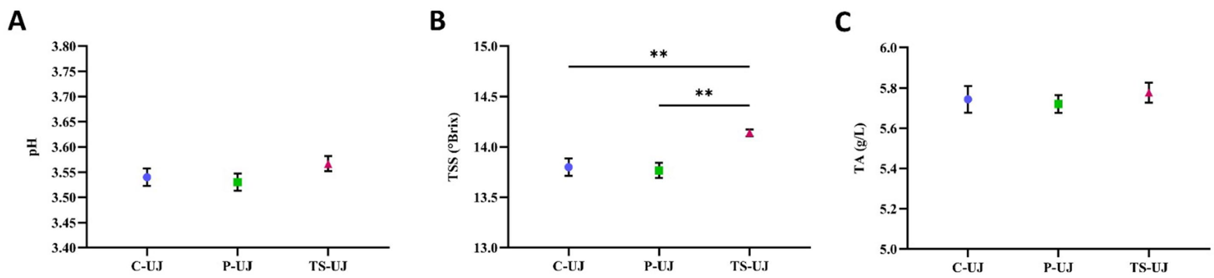

FIGURE 7
pH **(A)**, TSS: total soluble solids **(B)**, and TA: titratable acidity **(C)**. The symbols at the top of the bars indicate statistically significant differences (*p < 0.05). C-UJ: Untreated Uruset apple juice; P-UJ: Pasteurized Uruset apple juice; TS-UJ: Thermosonicated optimized pollen Uruset apple juice.

values ranging from 5.72 to 5.78 g/L, indicating that the process has a minimal effect on acidity, which is very important for flavor stability. Pearson's positive correlation coefficients among the physicochemical parameters (pH, TSS, and TA) were significantly correlated with myricetin (1), cinnamic acid (1), and ferulic acid (0.99). In parallel with our study, it was stated that the thermosonication process of anthocyanin-enriched tomato juice did not cause a significant change in pH value (Lafarga et al., 2019). However, the significant increase in TSS and TA values after thermosyncatıon may be due to the difference in the sample and the applied temperature–time combination. The findings that thermal pasteurization process did not affect the pH, TSS (% Brix), and TA (g/L) values of Uruset apple juice are similar to the results of studies conducted on physalis juice for the same purposes (Rabie et al., 2015).

3.4 Organic acid profile

The organic acid composition analysis of Uruset apple juice shows minor differences among control (C-UJ), pasteurized (P-UJ), and thermosonicated (TS-UJ) samples. Table 4 presents the results of organic acid analysis of C-UJ, P-UJ and TS-UJ Uruset apple juice. Total organic acid content is slightly higher in TS-UJ (1.33 ± 0.01 g/100 mL) compared to C-UJ (1.30 ± 0.02 g/100 mL) and P-UJ (1.25 ± 0.01 g/100 mL), but the differences are not statistically significant ($p > 0.05$). Among the individual organic acids, citric acid is the dominant component, followed by malic acid and tartaric acid, all showing minimal differences among the treatments. The citric acid content in TS-UJ (0.97 ± 0.013 g/100 mL) is significantly higher ($p < 0.05$) than in P-UJ (0.91 ± 0.01 g/100 mL), indicating that thermosonication can better preserve this organic acid. At the same time, pasteurization appears to cause slight deterioration. However, malic acid and tartaric acid levels remain unchanged in all treatments ($p > 0.05$), indicating that these acids are more stable under different processing conditions. As demonstrated in the study by Yıkmış et al. (2022), the implementation of thermosonication treatment on freshly squeezed pomegranate juice did not lead to a substantial change in the amount of malic acid, with a ($p < 0.05$) level of significance, similar to our study.

Although the observed changes were small, the slightly higher organic acid content in TS-UJ suggests that thermosonication may help preserve acidity, which may be beneficial for flavor, microbial stability, and antioxidant properties. The lower total organic acid content in pasteurized samples suggests that thermal treatment may lead to slight degradation of some acid components, potentially affecting sensory and preservation qualities of the juice. These findings suggest that thermosonication may be a preferable alternative to pasteurization in terms of preserving the natural acid composition of Uruset apple juice and preserving its freshness and stability.

3.5 Sugar profile

Sugar composition analysis of Uruset apple juice revealed significant differences among control (C-UJ), pasteurized (P-UJ), and thermosonicated (TS-UJ) samples. Sugar content is given in Table 4. Total sugar content was highest in TS-UJ (10.01 ± 0.00 g/100 mL), followed by C-UJ (9.90 ± 0.00 g/100 mL) and lowest in P-UJ (9.32 ± 0.04 g/100 mL) with statistically significant differences ($p < 0.05$) among all treatments. These results indicate that thermosonication may contribute to maintaining or even increasing sugar concentrations, while pasteurization decreases slightly. Unlike our study, Liao et al. (2020) found that ultrasonication treatment did not significantly affect the total sugar

TABLE 4 Organic acid, sugar, and phenolic compounds analysis results of C-UJ, P-UJ and TS-UJ.

Studied compound		Samples		
		C-UJ (µg/mL)	P-UJ (µg/mL)	TS-UJ (µg/mL)
Organic acid (g/100 mL)	Citric Acid	0.95 ± 0.013^{a}	0.91 ± 0.01^{a}	0.97 ± 0.013^{b}
	Malic Acid	0.29 ± 0.00^{a}	0.28 ± 0.00^{a}	0.29 ± 0.00^{a}
	Tartaric Acid	0.07 ± 0.00^{a}	0.06 ± 0.00^{a}	0.07 ± 0.00^{a}
	Total	8.77 ± 0.65	8.12 ± 0.47	8.69 ± 0.36
Sugars (µg/mL)	Fructose	5.69 ± 0.07^{b}	5.31 ± 0.03^{a}	5.74 ± 0.08^{b}
	Glucose	2.20 ± 0.03^{ab}	2.11 ± 0.02^{a}	2.24 ± 0.03^{b}
	Sucrose	1.90 ± 0.027^{b}	1.79 ± 0.02^{a}	1.92 ± 0.02^{b}
	Sorbitol	0.20 ± 0.00^{b}	0.19 ± 0.00^{a}	0.20 ± 0.00^{b}
	Total	9.90 ± 0.00^{b}	9.32 ± 0.04^{a}	10.01 ± 0.00^{c}
Phenolic compounds (µg/mL)	Klorogenic acid	61.43 ± 1.56^{ab}	55.83 ± 1.52^{a}	64.39 ± 0.91^{b}
	Gallic Acid	4.10 ± 0.03^{b}	3.73 ± 0.03^{a}	4.39 ± 0.07^{c}
	Caffeic acid	3.10 ± 0.05^{b}	2.81 ± 0.06^{a}	3.22 ± 0.05^{b}
	p-Coumaric acid	1.42 ± 0.04^{ab}	1.29 ± 0.04^{a}	1.57 ± 0.04^{b}
	Quercetin	13.26 ± 0.19^{b}	12.04 ± 0.20^{a}	13.79 ± 0.20^{b}
	Phloridzin	9.23 ± 0.13^{c}	8.40 ± 0.11^{a}	8.77 ± 0.12^{ab}
	Catechin	13.33 ± 0.29^{ab}	12.03 ± 0.41^{a}	13.86 ± 0.30^{b}
	Epicatechin	15.39 ± 0.33^{a}	14.07 ± 0.20^{a}	16.03 ± 1.15^{a}
	Cinnamic acid	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.01 ± 0.00^{b}
	Ferulic acid	0.23 ± 0.00^{a}	0.21 ± 0.00^{a}	0.47 ± 0.02^{b}
	Rutin	0.05 ± 0.00^{b}	0.00 ± 0.00^{a}	0.08 ± 0.00^{c}
	Myricetin	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.19 ± 0.00^{b}
	Total	121.28 ± 2.38^{b}	110.22 ± 2.37^{a}	126.59 ± 2.68^{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-UJ: Control Uruset juice; P-UJ: Pasteurized Uruset apple juice; TS-UJ: Thermosonicated optimized pollen Uruset apple juice.

content of clear red pitaya juice ($p > 0.05$). Among individual sugars, fructose, glucose, and sucrose are the main components, and fructose is the dominant sugar in all treatments. TS-UJ exhibits the highest fructose content (5.74 ± 0.08 g/100 mL), which is significantly higher than P-UJ (5.31 ± 0.03 g/100 mL) ($p < 0.05$), indicating that thermal and ultrasonic effects can increase sugar release or reduce degradation. Similarly, glucose and sucrose levels are slightly lower in pasteurized samples than control and thermosonic juices, indicating a small degradation effect during conventional heat treatment. Sorbitol content remains unchanged throughout the treatments, indicating that this sugar alcohol is more stable under different processing conditions. Statistical analysis showed that TS-UJ maintained significantly higher total sugar levels than P-UJ ($p < 0.05$). These findings highlight that thermosonication may be an effective method to preserve or improve the natural sugar profile of Uruset apple juice, making it a promising alternative to conventional pasteurization techniques. Ultrasound treatment provides mechanical effects through shear forces, allowing greater solvent penetration into the sample matrix (Mala et al., 2021).

3.6 Phenolic compounds profile

The phenolic compound result of Uruset apple juice is given in Table 4. The phenolic compound analysis of Uruset apple juice reveals significant differences among control (C-UJ), pasteurized (P-UJ) and thermosonicated (TS-UJ) samples. Total phenolic content was highest in TS-UJ (126.59 ± 2.68 μg/mL), followed by C-UJ (121.28 ± 2.38 μg/mL) and lowest in P-UJ (110.22 ± 2.37 μg/mL). There were statistically significant differences ($p < 0.05$) between pasteurized and thermosonicated samples. This result suggests that thermosonication may better preserve phenolic compounds than pasteurization, which has been shown to degrade certain bioactive compounds. Among the phenolic compounds, chlorogenic acid, quercetin, catechin, and epicatechin are found in relatively high concentrations and show significant differences depending on the processing method. For example, chlorogenic acid levels in TS-UJ (64.39 ± 0.91 μg/mL) are significantly higher ($p < 0.05$) than in P-UJ (55.83 ± 1.52 μg/mL), indicating that pasteurization leads to more significant loss of this compound. Similarly, quercetin and catechin levels are statistically higher in TS-UJ than P-UJ, supporting the hypothesis that thermosonication preserves flavonoids more effectively than conventional heat treatment. In a recent study, Erdal et al. (2022) discovered that p-coumaric acid and quercetin levels increased after ultrasound treatment. This outcome is consistent with the findings of this study. The level of caffeic acid was measured in the thermal pasteurised group (P-UJ) at 2.81 μg/mL, in the TS-UJ group at 3.22 μg/mL, and in the C-UJ group at 3.10 μg/mL. These differences are also statistically significant. Following the findings of the present study, the levels of caffeic and coumaric acids in black carrots were found to be comparatively low chlorogenic acid levels (Anandhi et al., 2024; Blando et al., 2021). Furthermore, pasteurization seems to have a reducing effect on most phenolic compounds, evidenced by significantly lower levels of gallate ($p < 0.05$), caffeic acid ($p < 0.01$), and phloridzin ($p < 0.05$) in P-UJ compared to TS-UJ and C-UJ. Interestingly, sinapic acid and myricetin were detected only in trace amounts in TS-UJ but not in the other samples, which may be due to structural changes induced by thermosonication. The presence of these compounds in TS-UJ but not in C-UJ suggests that ultrasound-induced cavitation and heat may facilitate the release or transformation of bound phenolic compounds. Statistical analysis highlights that TS-UJ showed significant differences ($p < 0.05$ or lower) compared to both C-UJ and P-UJ for most phenolic compounds. This suggests that thermosonication may preserve and enhance the extractability of certain phenolics. The moderate positive correlation ($r \approx 0.6–0.8$) between phenolic compounds and sensory properties (taste, odor and overall acceptability) indicates that thermosonication positively affects product quality and consumer appreciation. Particularly, compounds such as chlorogenic acid, quercetin and caffeic acid showed high positive correlation, indicating that thermosonication and pollen addition supported the extraction of these components. These findings highlight the strong influence of processing conditions on phenolic compound stability and suggest that thermosonication is a promising technology to preserve and potentially enrich the bioactive profile of Uruset apple juice.

3.7 Polyphenol oxidase enzyme activity

Polyphenol oxidase (PPO) activity in Uruset apple juice showed significant differences among control (C-UJ), pasteurized (P-UJ) and thermosonicated (TS-UJ) samples. PPO results are given in Figure 8. The highest PPO activity was observed in C-UJ (70.81%), while P-UJ showed the lowest activity (42.49%), indicating that pasteurization effectively inactivated PPO enzymes. TS-UJ showed a moderate value (54.52%), indicating that thermosensation led to a partial decrease in enzyme activity but was not as effective as pasteurization. The moderate inactivation in TS-UJ can be attributed to the combined thermal and cavitation effects of thermosonication, which disrupted enzyme structures while preserving more bioactive compounds than conventional heat treatments. The strong negative correlation ($r \approx -0.8$) between polyphenol oxidase (PPO) activity and phenolic compounds and antioxidant capacity indicates that inactivation of PPO contributes to preserving phenolic compounds and increasing antioxidant capacity. Similar to our study, Aadil et al. reported that thermosonication application caused a decrease in the PPO activity of grapefruit juice (Aadil et al., 2015). Considering that PPO is responsible for enzymatic browning, the decrease in its activity in P-UJ

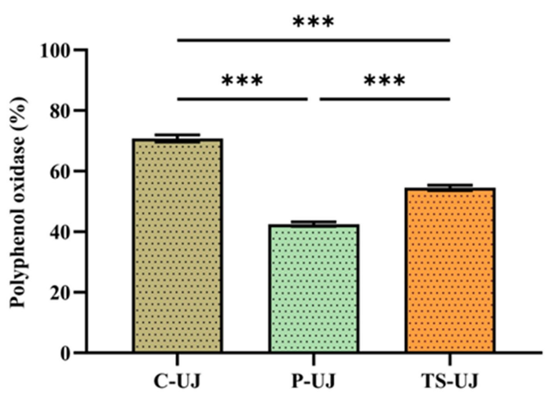

FIGURE 8
Polyphenol oxidase results of C-UJ, P-UJ and TS-UJ. C-UJ: Control Uruset apple juice; P-UJ: Pasteurized Uruset apple juice; TS-UJ: Thermosonicated optimized pollen Uruset apple juice.

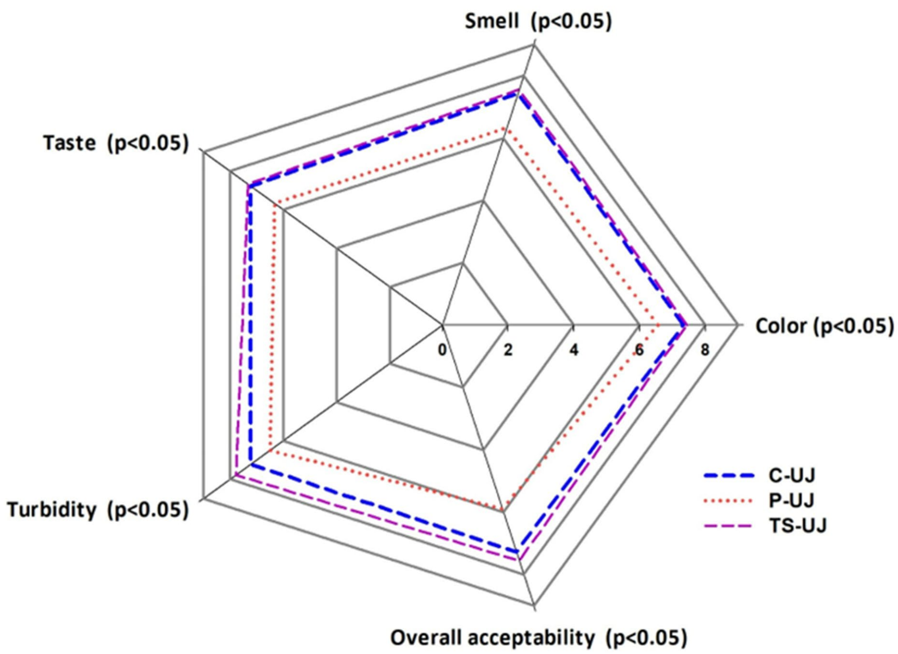

FIGURE 9
Sensory analysis results of C-UJ, P-UJ, and TS-UJ. C-UJ: Control Uruset apple juice; P-UJ: Pasteurized Uruset apple juice; TS-UJ: Thermosonicated optimized pollen Uruset apple juice.

and TS-UJ implies that both treatments can improve juice's visual appeal and shelf-life stability. The synergistic effect of temperature and ultrasound was more effective in denaturing PPO (Zhang et al., 2017).

3.8 Sensory properties

The sensory analysis results of Uruset apple juice are given in Figure 9. The sensory analysis results of Uruset apple juice revealed significant differences among control (C-UJ), pasteurized (P-UJ), and thermosonicated (TS-UJ) samples, highlighting the effect of processing methods on consumer perception. Color, odor, taste, turbidity, and overall acceptability scores were significantly lower in pasteurized samples (P-UJ), while thermosonicated samples (TS-UJ) generally maintained higher sensory attributes than the control group. Shen et al. (2021) observed that the overall liking of ultrasound-treated apple juice increased, which is similar to our study. In particular, the color and odor intensity of TS-UJ was rated significantly higher than P-UJ ($p < 0.05$), indicating that thermosonication better preserves the fresh and natural characteristics of the juice. In addition, the taste and overall acceptability of TS-UJ were statistically superior to P-UJ ($p < 0.05$), indicating that thermosonication may increase flavor perception and consumer preference compared to conventional heat treatment. The lower acceptability of P-UJ may be attributed to the thermal degradation of volatile compounds and bioactive components, leading to reduced sensory appeal. Moreover, turbidity, a critical quality parameter affecting consumer perception, was significantly higher in TS-UJ than P-UJ ($p < 0.05$), indicating that thermosonication preserves the natural turbidity of apple juice, which is generally associated with higher bioactive content. Tukey's HSD test revealed significant differences between the treatments, indicating that thermosonication maintains or even improves the sensory profile of Uruset apple juice compared to pasteurisation. These findings align with previous studies showing that nonthermal technologies can improve sensory properties while minimizing nutrient loss, making them a promising alternative for high-quality juice processing. The positive correlation observed between sensory properties and antioxidant capacity indicates that thermosonication process improves both nutritional and sensory quality. Taste showed a significant negative correlation with phloridzin (−0.82) and polyphenol oxidase (−0.83). These results show that thermosonication is an effective method for developing a functional beverage by optimizing the bioactive and sensory properties of Uruset apple juice. Given that sensory properties play an important role in consumer acceptance, the results support the potential application of thermosonication in the juice industry to improve product quality while maintaining the desired sensory attributes.

3.9 Principal component analysis (PCA)

PCA (Principal Component Analysis) plot visually summarizes the changes in sensory and chemical properties of Uruset apple juice samples due to different processing methods (control, pasteurization, and thermosonication) and the relationships between these properties. Two main components explain almost all of the total variance (100%) (first component 73.6%; second component 26.4%). The first principal component (PC1) shows a strong relationship with phenolic compounds (especially chlorogenic acid, gallic acid, catechin, quercetin), sugars (fructose, glucose, sorbitol), organic acids (malic acid, tartaric acid), total soluble solids (TSS), turbidity, polyphenol oxidase (PPO) and pH (Figure 10). This shows that PC1 mainly reflects chemical quality indicators and thermosonication treated samples (TS-UJ) are richer in these parameters. The control group (C-UJ) shows a higher enzymatic

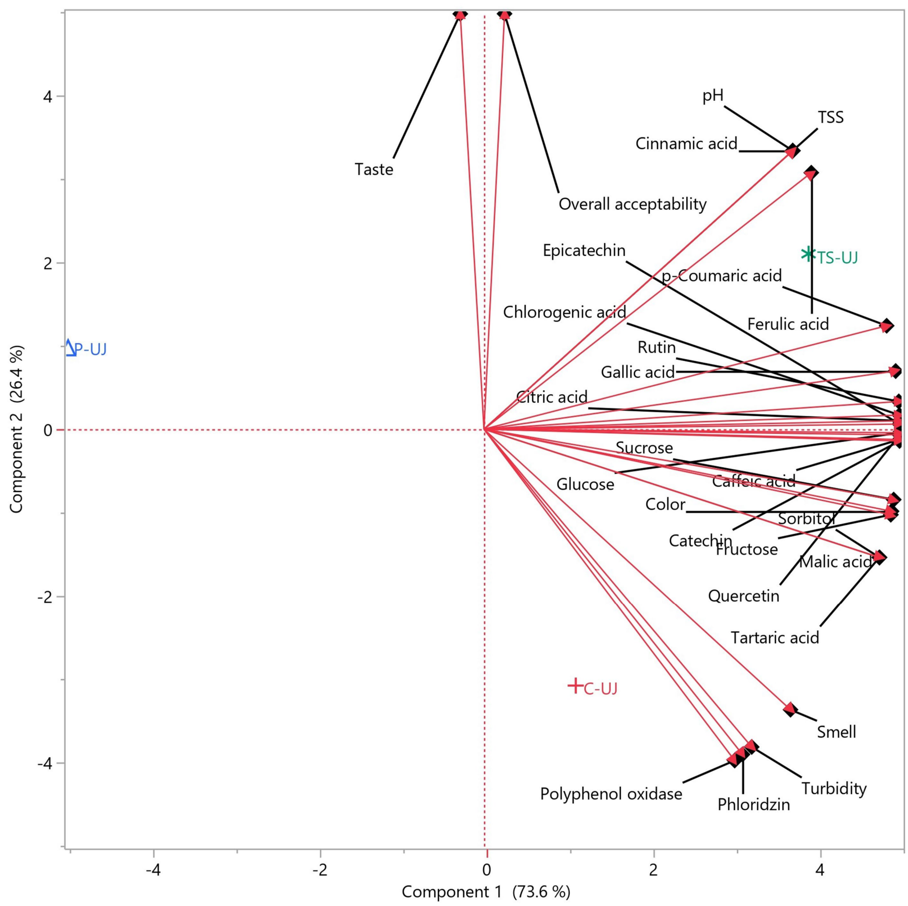

FIGURE 10
Principal component analysis (PCA) results on chemical and sensory properties of thermosonicated (TS-UJ), pasteurized (P-UJ) and control (C-UJ) Uruset apple juice samples.

activity, especially with a negative separation of polyphenol oxidase activity. The second component (PC2) in the graph explains 26.4% of the total variance and clearly represents sensory parameters (taste and general acceptability). Sensory parameters show a relatively independent distribution from other chemical properties. It is seen that thermosonicated samples (TS-UJ) are more sensory acceptable and preferable by the consumer. Thus, PCA results confirm that thermosonication is effective both in preserving and increasing bioactive components and in developing a product with high general acceptability. This situation reveals that thermosonication offers significant advantages compared to traditional methods for Uruset apple juice.

4 Conclusion

This study was conducted to optimize the bioactive and sensory properties of thermosonicated fortified-pollen Uruset apple juice. The thermosonication process and pollen addition increased the apple juice's total phenolic and flavonoid content and improved its antioxidant capacity. Especially phenolic compounds were preserved at higher levels and increased in quantity after thermosonication. The addition of pollen increased the stability of phenolic compounds and had a positive effect on antioxidant activity. The sensory analysis showed that the thermosonication process and pollen addition improved the apple juice's taste, odour, and general acceptability. The decrease in polyphenol oxidase activity contributed to preserving phenolic compounds. The synergistic effect of the thermosonication process and pollen addition was demonstrated by the positive correlation between sugar components and phenolic compounds. These results show that the thermosonication process and pollen content effectively produce functional beverages by improving the bioactive and sensory properties of Uruset apple juice. The study demonstrated the potential of valuable bioactive ingredients such as pollen in the beverage industry and supported the applicability of thermosonication in the production of functional beverages. These findings provide a scientific basis for the industrial processing of Uruset apple juice and the development of functional products.

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. BA: Writing – original draft, Writing – review & editing, Resources, Formal analysis. IMA: Writing – review & editing, Writing – original draft. MOA: Writing – original draft, Funding acquisition, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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