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Thermosonication Processing of Purple Onion Juice (*Allium cepa* L.): Anticancer, Antibacterial, Antihypertensive, and Antidiabetic Effects

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Abstract: Onion (*Allium cepa* L.) juice is an important product used in gastronomy and food formulations. The first objective of this study was to optimize the content of bioactive compounds in purple onion juice (POJ) after the thermosonication process using response surface methodology (RSM) and artificial neural network (ANN) application models. Second, the anticancer, antibacterial, antihypertensive, and antidiabetic effects of POJ obtained after thermal pasteurization (P-POJ) or thermosonication (TS-POJ) were investigated after obtaining the ANN and RSM analysis reports. The optimization process for TS-POJ was carried out at 44 °C, for 13 min, with a 68% amplitude. The findings demonstrated that the angiotensin-converting enzyme (ACE) inhibition level was greater in TS-POJ samples than in the untreated control (C-POJ) sample ($p > 0.05$). C-POJ, TS-POJ, and P-POJ exhibited the inhibition of cell proliferation in vitro in a dose-dependent manner in lung (A549), cervical (HeLa), and colon cancer cells following 24 h incubation. Thermosonication or thermal pasteurization did not markedly affect the cell proliferation of the examined cancer cells compared to the untreated control group. While no antibacterial effect was observed with low concentrations of samples, they showed an antibacterial effect at pure concentrations (100%). The thermosonication treatment for processing purple onion juice was successful in this study's results.

Keywords: purple onion; thermosonication; artificial neural network; anticancer; antibacterial

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1. Introduction

Nowadays, the global market is growing with increasing health awareness, demand for natural foods, vegetables and fruit juices, and major developments in food production [1,2]. Onion (*Allium cepa* L.) is a common vegetable that is widely consumed all over the world. Onions contain various phytochemicals. Onion chemical compounds are very abundant and have various pharmacological mechanisms to prevent disease. Phenolic and sulphur-containing compounds such as onion A, quercetin, cysteine sulphoxides and quercetin glucosides are the most important bioactive compounds. Thanks to these bioactive compounds, studies have shown that onions have various health functions. They are antioxidant, anti-obesity, anti-diabetic, anti-cancer, cardiovascular protective, antimicrobial, hepatoprotective, anti-inflammatory, respiratory protective, neuroprotective and digestive protective [3,4].

The thermosonication process is a new technology used to overcome heat treatment limitations associated with longer holding times at high temperatures, such as thermal pasteurization. At the same time, thermosonication is an innovative process for ensuring food safety with minimal changes in the nutritional and sensory quality of food and beverages. In many studies, it was determined that bioactive components increase in foods and beverages [5]. Furthermore, there is various research in the literature showing that ultrasound technology enriches the quality of juice, with examples including black carrot juice [6], fresh pomegranate juice [7], fresh verjuice [8], posotia (*Vitex negundo*) juice [9] and beetroot (*Beta vulgaris* L.) juice [10].

Response surface methodology (RSM) is preferred in many studies to indicate the level of importance of each factor through mathematical modelling and to analyze the interactions between factors affecting experimental response values. In research, artificial neural networks (ANNs) have been developed as a predictive tool with higher efficiency than RSM, providing flexibility and accuracy in experimental data fitting and non-linear correlation estimation [7,11,12]. As far as we know, there is no study in the literature on how thermosonication treatment of purple onion juice affects the content of its bioactive compounds using RSM and ANN optimization. In this study, the anticancer, antibacterial, ascorbic acid, antihypertensive and antidiabetic effects of purple onion juice treated with thermosonication and thermal pasteurization were compared.

2. Materials and Methods

2.1. Purple Onion Juice Processing

Purple onions (*Allium cepa* L.) grown in the Turkey/Thrace region were used as raw material in the research. Weeding was conducted for damaged and not rotten purple onions. Then, washing was performed. The outer skins of the purple onions were peeled. The pulp was obtained by crushing the purple onions in a blender (Waring Commercial Blender, 8011ES, Torrington, CT, USA) at low speed for 60 s. POJ was purified of coarse particles by means of a single layer of sterilized muslin. POJ was transferred to 100 mL sterile bottles and stored at -18 ± 1 °C for further experiments. In the study, untreated POJ was coded as control (C-POJ).

2.2. Thermal Pasteurization and Thermosonication Treatments

For the pasteurization of purple onion juice samples, samples (100 mL) were placed in sterile glass containers and left in a hot water bath (Wisd-Model WUC-D06H, Daihan, Wonju, Republic of Korea) at 85 °C for 2 min. The temperature and time intervals were determined based on previously reported results [7]. The thermally pasteurized sample was coded pasteurized purple onion juice (P-POJ). For thermosonication (TS) treatment, 100 mL of POJ was processed using an ultrasonic processor (Hielscher Ultrasonics model UP200St, Berlin, Germany) at a frequency of 26 kHz and 200 W. The ultrasound parameters studied were amplitude (60%, 70%, 80%, 90% and 100%), treatment time (8, 10, 12, 14 and 16 min) and temperature (40, 45, 50, 55 and 60 °C) in constant mode. To prevent overheating during ultrasonic processing, an ice water bath was used. After the TS, the POJ samples (TS-POJ) were immediately cooled in an ice bath and kept at -18 ± 1 °C until the analysis was performed.

2.3. Modelling Procedure for Response

To understand the effect of thermosonication treatments on bioactive compounds in purple onion juice, Response surface methodology (RSM) and artificial neural networks (ANNs) were used. Amplitude (X_3, 60–100%), temperature (X_1, 40–60 °C) and time (X_2, 8–16 min) were independent factors. Antioxidants, total flavonoids (TFC; mg CE/L), total phenolics (TPC; mg GAE/L), DPPH (% inhibition), CUPRAC (% inhibition) and total anthocyanins (TAC; mg mv-3-glu/L) were response variables. For RSM, a central composite design (CCD) was implemented using Minitab software (version 19, Minitab Software, State College, PA, USA) for the optimization of thermosonication processing

of purple onion juice. The number of experiments in the CCD design was 20, performed in triplicate. The results are presented in Table 1. In the equation models, the following quadratic polynomial formula was used:

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

The definition of this formula consists of the dependent variable (y); the intercept term (β_0); the coefficient of the first (linear) equation (β_i); the coefficient of the second (quadratic) equation (β_{ii}); the coefficient of the two-factor interaction (β_{ij}); and the independent variables X_i and X_j.

ANN was implemented using the MATLAB Neural Network Toolbox (MATLAB version R2020b—Mathworks Inc., Natick, MA, USA) for the optimization of the processing of pure onion juice by thermosonication. The parameters of the model obtained in our previous study were used [13]. The results of the ANNs for TPC, TFC, DPPH, CUPRAC, and TAC were examined. The learning rates were set to 1.7799, 4.081, 0.87261, 0.00032 and 0.0018, respectively. The ANNs were trained with 1000 iterations. Epochs 103, 2, 5, 107 and 67 were the best performing epochs for the responses. The performance of the system was measured using the plot regression function. The generated neural network input, hidden and output layers were different for each bioactive component response (Figure 1).

The coefficient of determination (R^2), root mean square error (RMSE) and absolute average deviation (AAD) between the RSM and ANN models were compared in order to clarify the performance of the models. The formulas are as follows:

$$R^2 = 1 - \frac{\sum_{i=1}^{n} \left(Y_{Predicted} - Y_{Experimental}\right)^2}{\sum_{i=1}^{n} \left(Y_{Average} - Y_{Experimental}\right)^2} \quad (2)$$

$$RSME = \left(\frac{1}{n} \sum_{i=1}^{n} \left(Y_{Predicted} - Y_{Experimental}\right)^2 \right)^{\frac{1}{2}} \quad (3)$$

$$AAD = \left(\frac{1}{n} \sum_{i=1}^{n} \left| \frac{Y_{Predicted} - Y_{Experimental}}{Y_{Experimental}} \right| \right) * 100 \quad (4)$$

where n, $Y_{Average}$, $Y_{Predicted}$, and $Y_{Experimental}$ are the number of data points, the average of data, the predicted value, and the experimental value, respectively. The accuracy and validity of the models were measured on the basis of R^2, AAD and RMSE.

2.4. Determination of Bioactive Compounds

The Folin–Ciocalteu method was used to determine the TPC (total phenolic content) as follows [14]. The results are expressed in mg gallic acid equivalents per liter. Total flavonoid content (TFC) was determined by calorimetric method [15]. Results were expressed as mg catechin equivalents per L. Total monomeric anthocyanin content (TAC) was determined by the pH difference method [16,17]. Absorbances were measured at 528 nm ($\lambda_{vis\text{-}max}$). Results were expressed as mg malvidine-3-O-glucoside (mv-3-glu) equivalents per liter of juice. The DPPH (2,2-diphenyl-1-picrylhydrazyl) method, in which the DPPH radical is formed on the basis of inhibition, was used with some modifications for the determination of antioxidant capacity [18]. The Cu(II) ion Reducing Antioxidant Capacity (CUPRAC) method was used to determine the antioxidant capacity [19]. Each POJ sample was analyzed in triplicate.

Table 1. Experimental and predicted responses of RSM and ANN and results for C-POJ, P-POJ and TS-POJ.

Run No	Independent Variables			Dependent Variables													
				TPC (mg GAE/100 mL)			TFC (mg CE/100 mL)			DPPH (% Inhibition)			CUPRAC (% Inhibition)			TAC (mg of mv-3-glu/L)		
	Tempature (°C-X_1)	Time (min-X_2)	Amplitude (%-X_3)	Experimental Data	RSM Prediction	ANN Prediction	Experimental Data	RSM Prediction	ANN Prediction	Experimental Data	RSM Prediction	ANN Prediction	Experimental Data	RSM Prediction	ANN Prediction	Experimental Data	RSM Prediction	ANN Prediction
1	50	16	80	152.26 ± 1.95	152.24	153.12	20.85 ± 1.03	20.94	20.85	53.37 ± 0.54	53.95	53.37	56.28 ± 0.66	56.38	56.87	12.57 ± 0.20	12.93	12.57
2	50	12	80	160.14 ± 1.71	160.08	160.36	17.85 ± 0.54	17.88	17.85	44.63 ± 0.71	45.08	44.63	46.70 ± 0.81	46.88	46.71	10.31 ± 0.79	10.46	10.31
3	45	10	90	153.83 ± 0.55	154.29	153.90	21.98 ± 0.79	21.79	21.98	54.92 ± 0.42	54.60	54.92	57.15 ± 0.10	56.81	57.13	11.70 ± 0.65	11.68	11.59
4	50	12	100	147.74 ± 1.95	147.57	147.83	21.11 ± 1.51	21.23	21.11	49.20 ± 1.36	50.11	49.20	54.88 ± 0.40	55.17	55.01	12.19 ± 0.51	12.32	12.19
5	50	12	80	160.80 ± 1.22	160.08	160.36	17.85 ± 1.13	17.88	17.85	44.63 ± 0.72	45.08	44.63	46.70 ± 0.59	46.88	46.71	10.31 ± 0.13	10.46	10.31
6	55	14	90	156.13 ± 2.14	156.24	156.22	22.30 ± 0.41	21.91	22.30	52.94 ± 0.86	52.17	52.94	55.66 ± 1.27	55.24	55.66	12.89 ± 0.64	12.74	12.89
7	45	10	90	153.95 ± 2.60	154.29	153.90	21.99 ± 1.07	21.79	21.98	54.98 ± 0.34	54.60	54.92	57.22 ± 0.37	56.81	57.13	11.48 ± 0.37	11.68	11.59
8	50	12	80	160.14 ± 1.68	160.08	160.36	17.85 ± 0.28	17.88	17.85	44.63 ± 0.62	45.08	44.63	46.70 ± 1.15	46.88	46.71	10.31 ± 0.24	10.46	10.31
9	50	12	80	160.14 ± 1.53	160.08	160.36	17.85 ± 0.40	17.88	17.85	44.63 ± 0.57	45.08	44.63	46.70 ± 0.66	46.88	46.71	10.31 ± 0.44	10.46	10.31
10	55	14	70	147.36 ± 2.12	147.30	147.37	21.05 ± 1.80	20.84	21.05	52.63 ± 0.72	52.71	52.63	54.74 ± 0.85	54.32	54.66	12.16 ± 0.10	12.07	12.15
11	50	8	80	145.43 ± 1.34	144.54	145.11	19.68 ± 0.31	19.95	19.68	53.85 ± 0.89	55.07	53.85	56.27 ± 0.21	57.29	56.08	12.00 ± 0.62	12.10	12.02
12	50	12	80	160.14 ± 2.59	160.08	160.36	17.85 ± 0.74	17.88	17.85	44.63 ± 0.33	45.08	44.63	46.70 ± 0.34	46.88	46.71	10.31 ± 0.16	10.46	10.31
13	60	12	80	141.04 ± 1.36	140.91	140.82	20.15 ± 0.52	20.42	20.15	51.44 ± 1.02	52.54	51.44	53.39 ± 0.47	54.10	53.27	11.94 ± 0.92	12.27	11.94
14	45	10	70	149.62 ± 3.38	150.29	149.66	21.37 ± 0.88	21.20	21.37	53.28 ± 0.35	53.19	53.28	55.57 ± 0.47	54.84	55.52	12.48 ± 0.21	12.59	12.46
15	50	12	60	135.37 ± 1.10	134.63	135.25	19.34 ± 1.05	19.58	19.34	48.35 ± 1.39	49.24	48.35	51.45 ± 1.24	52.28	51.41	12.22 ± 0.88	12.56	13.07
16	40	12	80	150.45 ± 3.93	149.66	148.32	21.54 ± 0.45	21.64	21.54	53.64 ± 0.76	54.34	53.64	54.88 ± 1.03	55.29	54.84	12.42 ± 0.17	12.56	12.41
17	45	14	70	156.85 ± 0.83	157.58	156.91	21.64 ± 0.34	21.31	21.64	56.02 ± 0.75	55.35	55.66	57.26 ± 0.41	56.82	57.25	13.90 ± 0.18	13.70	13.90
18	55	10	90	155.77 ± 0.66	155.82	155.90	21.25 ± 1.16	21.02	21.25	55.63 ± 1.06	55.44	54.98	58.85 ± 0.52	58.13	58.71	12.86 ± 0.31	13.02	12.93
19	55	10	70	125.94 ± 2.29	126.75	127.34	15.27 ± 0.30	14.76	15.27	41.65 ± 0.57	40.58	43.27	44.42 ± 0.24	43.26	46.01	9.85 ± 0.52	9.71	9.85
20	50	12	80	160.14 ± 1.56	160.08	160.36	17.85 ± 0.89	17.88	17.85	44.63 ± 0.71	45.08	44.63	46.70 ± 0.81	46.88	46.71	10.31 ± 0.25	10.46	10.31
Predictive capacity comparison of RSM and ANN models for five response variables				R^2	0.99	0.99		0.99	1.00		0.98	0.99		0.99	0.99		0.97	0.97
				RMSE	0.28	0.63		0.22	0.00		0.67	0.40		0.54	0.15		0.19	0.19
				AAD (%)	0.24	0.24		0.79	0.01		1.19	0.29		1.19	0.29		0.23	0.12
TS-POJ	44	13	68		156.85			22.51			57.50			59.05			14.38	
C-POJ					147.22			19.64			54.68			55.84			15.77	
P-POJ					142.86			17.78			52.33			53.35			12.86	

TAC: total anthocyanin content; TFC: total flavonoid content; TPC: total phenolic content; GAE: gallic acid equivalent; CE: catechin equivalent; mv-3-glu: malvidin 3-monoglucoside; C-POJ (untreated purple onion juice); P-POJ (thermal pasteurized purple onion juice); TS-POJ (thermosonication-treated purple onion juice). RSM: response surface methodology; R^2: coefficient of determination; RMSE: root mean square error; AAD: absolute average deviation; ANN: artificial neural network. The results are presented as mean ± standard deviation (n = 3).

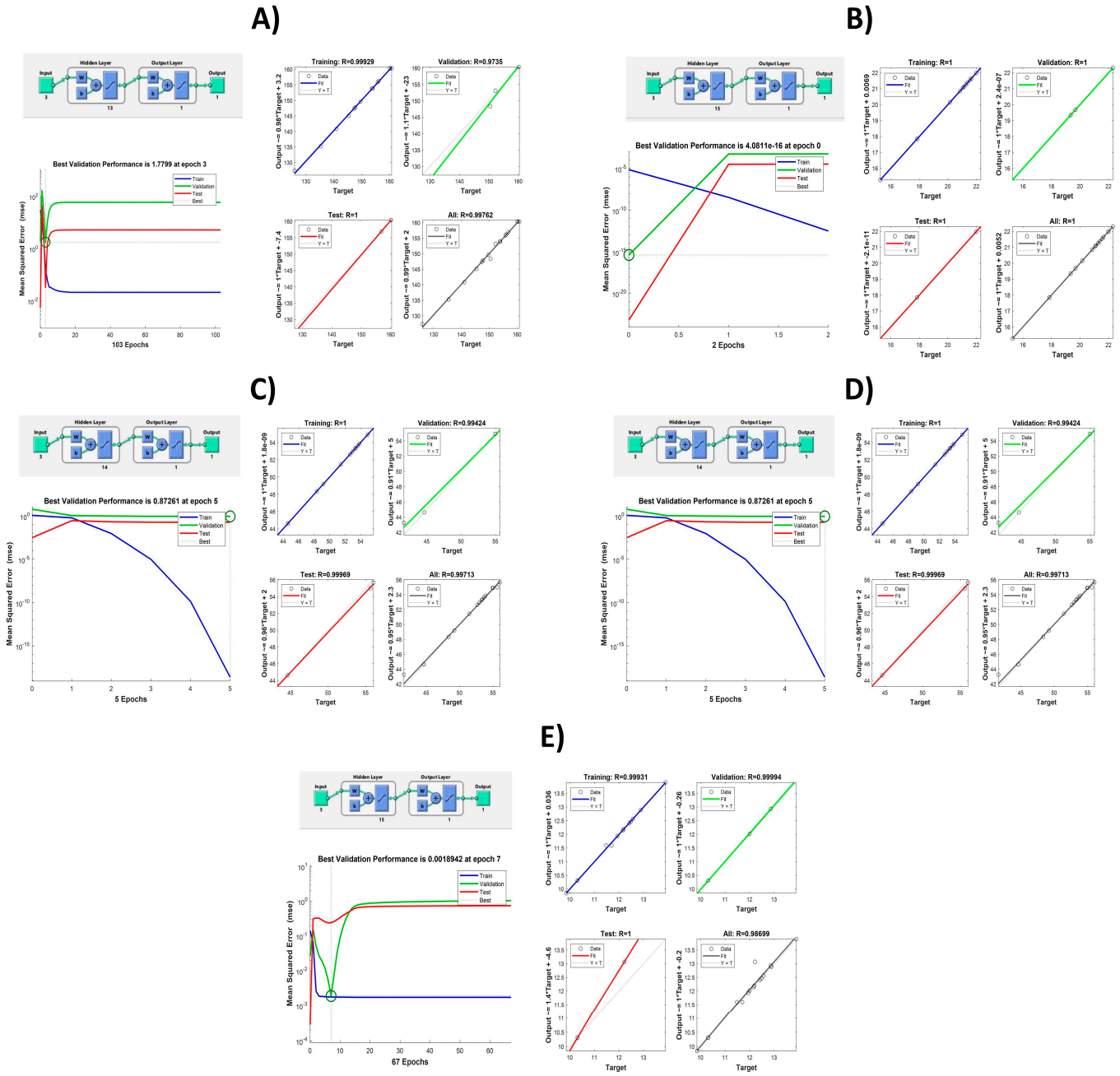

Figure 1. (**A**) TPC, (**B**) TFC, (**C**) DPPH, (**D**) CUPRAC, and (**E**) TAC, the optimal architecture of the developed ANN model, ANN model performance plot, actual versus predicted values using ANN.

2.5. Anticancer Activity

The lung (A549), cervical (HeLa), and colon (Caco-2) cancer cell lines were previously obtained from the American Type Culture Collection (ATCC). Dulbecco's modified eagle's medium (DMEM; Multicell, Wisent, St-Bruno, QC, Canada) containing 15% fetal bovine serum (Capricorn Scientific, Ebsdorfergrund, Germany), 1% nonessential amino acids (Corning™ cellgro™), sodium pyruvate (Corning™ cellgro™), and a penicillin/streptomycin antibiotic mixture (Corning™ cellgro™) was used for cultivation of cells at 37 °C in 5% CO_2 and 95% humidity environment. To determine the cytotoxic effects of C-, P and TS-POJ samples, the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed. Briefly, for each cancer cell line, approximately 1×10^5 cells/well were seeded in each 96-well culture plate and allowed to grow for 24 h. After this time, the cultivation medium of each well was replaced, and cells were admin-

istered C-POJ, TS-POJ, and P-POJ samples at different concentrations (80, 240, 480 and 720 µL/mL) sterilized by filtration (0.45 µm pore size). Following 24 h incubation, the MTT assay was performed, and then the optical density (OD) value of each well was determined using a microplate reader (Shimadzu: UV-2600 Spectrophotometer) at a wavelength of 570 nm as previously described [20]. The percentage of cell viability was determined using the following formula: % cell viability = (OD value of treated sample-OD value of blank/OD value of control-OD value of blank) × 100. The half maximal inhibitory concentration (IC_{50}) value was calculated using the Graphpad Prism 6.0 program.

2.6. Antibacterial Activity

Antibacterial activity of POJ samples was determined using the Clinical and Laboratory Standards Institute (CLSI) recommended Kirby–Bauer disc diffusion method [21]. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 11778, *Proteus vulgaris* ATCC 3851, *Pseudomonas aeruginosa* ATCC 27853 (Gram-negative bacteria) and *Enterococcus faecalis* ATCC 29212 (Gram-positive bacteria) were used as standard microorganisms. Bacterial strains were inoculated on 5% sheep blood agar (catalogue number: HM-09912; BES-LAB, Ankara, Türkiye) and then activated by incubation at 37 °C for 18–20 h. At the end of the incubation period, the bacterial density was adjusted to 0.5 MacFarland on Mueller Hinton broth (catalogue number: 4017412; Biolife, Milano, Italia) for all microorganisms. The adjusted bacterial suspension was inoculated on MH agar (catalogue number: 105437; Merck, Darmstadt, Germany). Whatman disks No. 3 (6 mm diameter) were impregnated with 100 µL of the samples (C-, P- or TS-POJ) and placed onto the surface of the inoculated plates. Plates were then incubated at 37 °C for 18–20 h. Gentamicin (CN, 10 µg, catalogue number: CT0024B; Oxoid, Thebarton, Australia) discs were used as a positive control. The diameters of the inhibition zones (mm) were measured at the end of the incubation period. Each test was repeated in triplicate.

2.7. Angiotensin-Converting Enzyme (ACE) Inhibitory and Antidiabetic Effects

For the antihypertensive activity, the assay of the ACE inhibitory activity was carried out with some modifications as follows [22]. For the determination of ACE inhibitory activity, the reaction mixture contained 50 µL of 8 mM HHL, 50 µL of the sample solution, and 100 µL of the ACE solution (2.5 mU/mL) as a substrate (Incubation for 90 min at 37 °C). The reaction was terminated by the addition of 250 µL of 1 N HCl. In an aqueduct, hippuric acid was redissolved. The absorbance of the sample of vinegar was measured using a UV/VIS spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Melbourne, Australia) at 228 nm. The IC50 concentration was used to determine the achievement of a 50% reduction in ACE activity. The anti-diabetic activity of the juice (alpha-glucosidase and alpha-amylase) was determined according to the modified method described below [23]. Acarbose was used as positive control in antidiabetic analyses. Absorbance measurements were performed by UV/VIS spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Melbourne, Australia).

2.8. Determination of Ascorbic Acid

The ascorbic acid content of purple onion juice was determined using 2,6-dichloroindophenol titration based on the method described in AOAC (1990) [24]. Ascorbic acid content was expressed as mg ascorbic acid/100 mL of juice. Three replicates were performed for each analysis.

2.9. Statistical Analysis

Data were analyzed using SPSS (v22.0, SPSS Inc., Chicago, IL, USA) and GraphPad (v6.0, GraphPad Software, San Diego, CA, USA). Experiment results are expressed as mean ± standard deviation (SD) of three replicates per treatment. One-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test was used to analyse the data. For the anticancer activity assays, a two-way ANOVA test was performed

using GraphPad to determine the interaction between the dependent (cell viability) and independent (type and cellular dose of POJ samples applied to Caco-2, A549, or HeLA cells) variables. If a significant 2-way interaction was found, then the significance of differences between pairs of group means were determined using Tukey's post hoc test. Data for cell viability are expressed as the mean ± SEM for at least three replicates ascertained in three independent experiments. The statistical significance for all tests was set at $p < 0.05$.

3. Results

3.1. Optimization of TPC, TFC, DPPH, CUPRAC, and TAC Assays/Determinations

Table 1 shows the experimental and predicted results for TPC, TFC, DPPH, CUPRAC, and TAC values for TS-POJ samples with different amplification levels, temperatures, and times. The model was in good agreement with the experimental results as the R^2 and R^2 values were above 98%. The equations below show the effects of three independent variables (temperature, time, and amplitude) on POJ bioactivity.

$$\mathrm{TPC(mgGAE/100mL)} = -175.0 + 0.340X_1 + 22.04X_2 + 4.664X_3 - 0.14787X_1^2 - 0.7304X_2^2 - 0.04744X_3^2 + 0.3316X_1X_2 + 0.12538X_1X_3 - 0.2516X_2X_3 \quad (5)$$

$$\mathrm{TFC(mgCE/100mL)} = 299.5 - 7.279X_1 - 6.012X_2 + 1.611X_3 + 0.03149X_1^2 + 0.1606X_2^2 + 0.006319X_3^2 + 0.1495X_1X_2 + 0.02842X_1X_3 - 0.06498X_2X_3 \quad (6)$$

$$\mathrm{DPPH(Inhibition)} = 650.6 - 16.82X_1 - 11.34X_2 - 2.869X_3 + 0.08357X_1^2 + 0.5892X_2^2 + 0.01148X_3^2 + 0.2492X_1X_2 + 0.06728X_1X_3 - 0.1925X_2X_3 \quad (7)$$

$$\mathrm{CUPRAC(Inhibition)} = 666.5 - 15.753X_1 - 12.44X_2 - 3.797X_3 + 0.07812X_1^2 + 0.6221X_2^2 + 0.01710X_3^2 + 0.2268X_1X_2 + 0.06453X_1X_3 - 0.1743X_2X_3 \quad (8)$$

$$\mathrm{TAC(mg}mv-3-glu\mathrm{/100mL)} = 239.7 + 5.459X_1 - 0.86X_2 - 0.260X_3 - 0.03362X_1^2 + 0.4518X_2^2 + 0.001995X_3^2 - 0.1746X_1X_2 - 0.00523X_1X_3 + 0.0201X_2X_3 \quad (9)$$

Table 1 also shows the experimental and predicted responses of RSM and ANN and the results for C-POJ and P-POJ. RSM modelling was carried out to determine optimal values in order to evaluate the effects of the independent variables on the bioactive compounds (TPC, TFC, DPPH, CUPRAC, and TAC). In particular, increasing the treatment duration and its amplitude had a positive effect on the TPC, TFC, DPPH, and CUPRAC results, which is consistent with previous studies on bioactive compound enrichment [25,26]. Numerical optimization was carried out to find the optimal combination of temperature, time, and amplitude values. This was performed to obtain the maximum bioactive yield of purple onion. By keeping the independent variables within the required ranges, the bioactive enrichment of POJ was also maximized. Optimal combinations were obtained after thermosonication at 44 °C, 13 min and 68% amplitude. Under these conditions, TPC, TFC, DPPH, CUPRAC, and TAC were 156.85 mg GAE/100 mL, 22.51 mg CE/100 mL, 57.50%, 59.05%, and 14.38 mg mv-3-glu/L, respectively. The highest increase was detected in the TFC values with 12.7% compared to the C-POJ sample in TS-POJ. Thermo-sonication treatment was superior to thermal pasteurization for enrichment and preservation of bioactive compounds. Similar superiority was also reported for black carrot juice [6], fresh pomegranate juice [7] and beetroot (*Beta vulgaris* L.) juice [10]. The enrichment of bioactive compounds can be attributed to the increase in cell wall permeability due to the combination of cavitation and temperature caused by thermosonication treatment.

The anthocyanins were degraded by the thermosonication and thermal pasteurization parameters. However, thermosonication protected more anthocyanins than thermal pasteurization. Thermal pasteurization resulted in a reduction anthocyanin value compared to the C-POJ sample. Li et al. (2022) found that red radish had increased antioxidants in their study of different thermosonication treatments, but there were decreases in anthocyanins [27].

The estimated values and experimental values for all responses for the optimal thermosonication conditions and verification are shown in Table 1. According to the validation results, slight variations between the experimental response and the values estimated from the optimum conditions revealed the validity of the optimum thermosonication conditions. Therefore, it was concluded that the model prediction for the optimization of thermosonication conditions for bioactive components (TPC, TFC, DPPH, CUPRAC, and TAC) can be applied in practice. The higher R^2 value of the ANN model in comparison with the RSM model, valid for all bioactive components, is an indication of the better prediction ability and accuracy of the ANN model. The TPC, TFC, DPPH, CUPRAC, and TAC values were 0.63, 0.00, 0.4, 0.08, 0.15, and 0.19, respectively, when examining the RSME values for the ANN model. The RSME values for the ANN model were lower than those for the RSM predictions for TFC, DPPH, and CUPRAC. When the AAD values for the ANN model were examined, the TPC, TFC, DPPH, CUPRAC, and TAC values were measured as 0.24, 0.01, 0.29, 0.29, and 0.12, respectively. In general, the ANN model had better AAD results than the RSM model. The results of the whole modeling showed that both models had good predictive ability and accuracy; however, it was determined that the ANN model is superior to the RSM model in terms of comparison parameters. There are studies that have demonstrated the superiority of ANN vs. RSM, as in our research [11,12].

3.2. Anticancer Effects

A significant two-way interaction effect ($p = 0.048$) was found between treatment and POJ dose in regard to cell viability for the A549 cell line (Figure 2A). Treatment of A549 cells with C-, TS-, or P-POJ for 24 h led to a significant decrease in cell survival rate in a dose-dependent manner. The lowest (80 µL/mL) and highest (720 µL/mL) concentrations used for treatments caused approximately 25–30% and 70% cell death, respectively. When the A549 cells were exposed to the lowest (80 µL/mL) or highest (720 µL/mL) concentrations of POJ samples, regardless of their preparation method, the survival rate of the cells was markedly ($p < 0.0001$) decreased to approximately 30% and 70%, respectively, in comparison to the untreated control groups. The cytotoxic effect of TS-POJ on A549 cells was significantly lower than that observed for C-POJ ($p = 0.0001$) and TS-POJ ($p = 0.0472$) at the concentration of 240 µL/mL. The results demonstrated that POJ had an inhibitory effect on lung cancer cell proliferation in vitro, and the IC_{50} was 100.34, 130.48, and 120.75 µL/mL for C-, TS-, and P-POJ, respectively.

Unlike for A549 cells, no significant interaction effect of treatment and dose on survival rate was revealed for HeLa ($p = 0.4359$) and Caco-2 ($p = 0.5813$) cells (Figure 2B,C). On the other hand, treatment and dose main effects were noted for cell proliferation in HeLa ($p = 0.0485$; $p < 0.0001$, respectively) and Caco-2 cells ($p = 0.0232$; $p < 0.0001$, respectively). The anticancerogenic effect of P-POJ on HeLA cells was markedly lower than that of TS-POJ ($p = 0.0181$) at the concentration of 240 µL/mL (Figure 2B). Cell survival rate was significantly lower for Caco-2 cells subjected to C-POJ than TS-POJ ($p = 0.0415$) and P-POJ ($p = 0.0259$) at the 80 µL/mL dose concentration. The highest and lowest administrated concentration of the treatments led to a dramatic decrease (~65%; $p < 0.0001$ and ~20–25%; $p < 0.0001$ for C-POJ and TS-POJ, $p = 0.0017$ for P-POJ, respectively) in the survival of HeLa and Caco-2 cells. In HeLa cells, IC_{50} for C-POJ, TS-POJ, and P-POJ was determined to be 130.52, 150.48, and 220.26 µL/mL, respectively. For Caco-2 cells, IC_{50} values were 100.16, 130.80, and 130.67 µL/mL for C-POJ, TS-POJ, and P-POJ, respectively.

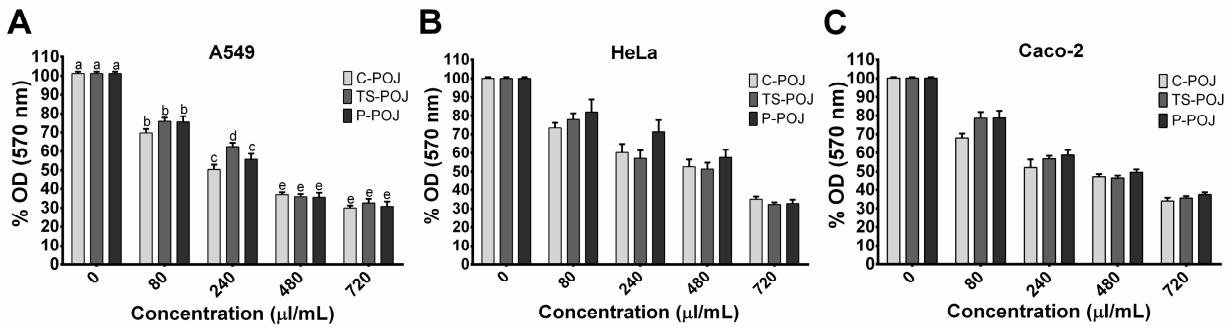

Figure 2. Anticancerogenic effects of purple onion juices obtained through thermal pasteurization and the thermosonication process. The MTT assay was performed to determine the cell viability in A549 (**A**), HeLa (**B**), and Caco-2 (**C**) cells following 24 h incubation in the presence of various concentrations (80, 240, 240, and 720 µL/mL) of C-, TS-, and P-POJ. Data expressed as the mean ± SEM of three biological replicates ascertained in three independent experiments were analyzed by two-way ANOVA. A significant 2-way interaction was found for treatment × dose ($p = 0.048$) in the A549 cell. Multiple pairwise comparisons were performed using Tukey's post hoc test. Different letters atop bars indicate statistically significant differences ($p < 0.05$). Treatment and cell proliferation were found significant in HeLa ($p = 0.048$ and $p < 0.001$, respectively) and Caco-2 cells ($p = 0.023$ and $p < 0.001$, respectively). OD: optical density; C-POJ (untreated purple onion juice); P-POJ (thermal pasteurized purple onion juice juice); TS-POJ (Thermosonication-treated purple onion juice).

In agreement with the outcomes of the current study, Zhou et al. (2020) demonstrated that extracts obtained from storey onion (*Allium cepa* L. var. *proliferum* Regel) and other common *Allium* spp. (onion and Welsh onion) exhibited a proliferative inhibition effect in a hepatocellular carcinoma cell line (HepG2) by triggering apoptosis of cells in a dose- and time-dependent manner [28]. A previous study report published by Yıkmış et al. (2022) revealed that purple onion vinegar samples prepared using traditional, pasteurization or ultrasound approaches had anti-proliferation effects on gastric (HGC-27) and colon (HCT-116) carcinoma cell lines [29]. It was also noted that the differences in the preparation method of purple onion vinegar had no significant effect on HGC-27 cell viability. Nile et al. (2021) reported in a study that onion spiraeoside and quercetin obtained from onion (*Allium cepa* L.) solid waste had anticancer activity against HeLa cells [30]. Another study report revealed that the survival rate of HT-29 cells (human colon carcinoma cell line) was significantly reduced following 24 h incubation of cells with ethanol extract of yellow onion (*Allium cepa* L.) peel in a dose-dependent manner [31]. It has been demonstrated that a subfraction of the red onion (*Allium cepa* L.) peel ethanolic extract had a similar anti-cancerogenic activity against human breast cancer cells (MDA-MB-231) [32].

3.3. Antibacterial Effects

While the uncontrolled use of antibiotics causes the emergence of multi-drug-resistant strains, it also has negative effects on microbiota. In recent years, alternative herbal treatment methods were investigated for the treatment of infectious diseases due to their protective properties for microbiota and not causing antibiotic resistance [33]. In this study, antibacterial effects of C-POJ, P-POJ and TS-POJ at concentrations varying between 3.125 and 100% were determined by the disc diffusion method. At the end of the study, the antibacterial effect was observed only with 100% concentrations of POJ samples despite the preparation method used in each case. The highest zone diameter was seen in *Proteus vulgaris* compared to other bacteria in all POJ samples. This finding suggests that it can be used as a support to antimicrobial treatment in infections caused by *Proteus vulgaris.* However, no difference was found between the inhibition zone diameters for Gram-negative (*Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) and Gram-positive (*Bacillus cereus*, *Staphylococcus aureus* and *Enterococcus faecalis*) bacteria (Table 2 and Figure 3).

Table 2. Inhibition zone diameters (mm) for Gram-positive and Gram-negative bacteria tested with C-POJ, P-POJ and TS-POJ.

Bacteria	Inhibition Zone Diameter (Mean ± SD, mm)								
	C-POJ			P-POJ			TS-POJ		
	%100	%50	CN	%100	%50	CN	%100	%50	CN
Escherichia coli	7.11 ± 0.08	ND	22.04 ± 1.05	7.02 ± 0.19	ND	23.00 ± 0.12	7.00 ± 0.20	ND	20.97 ± 0.45
Proteus vulgaris	10.00 ± 0.20	6.98 ± 0.13	21.74 ± 0.54	9.14 ± 0.13	7.13 ± 0.12	21.03 ± 0.15	9.00 ± 0.12	ND	21.95 ± 0.19
Pseudomonas aeruginosa	9.00 ± 0.12	ND	21.96 ± 0.2	8.03 ± 0.10	ND	21.02 ± 0.28	8.07 ± 0.50	ND	22.00 ± 0.06
Bacillus cereus	7.18 ± 0.21	ND	22.27 ± 0.51	7.11 ± 0.21	ND	21.67 ± 0.71	8.00 ± 0.12	ND	22.87 ± 0.37
Staphylococcus aureus	7.98 ± 0.53	ND	21.80 ± 0.30	8.05 ± 0.24	ND	23.00 ± 0.50	8.00 ± 0.12	ND	22.00 ± 0.60
Enterococcus faecalis	7.08 ± 0.17	ND	17.00 ± 0.15	6.91 ± 0.28	ND	17.03 ± 0.08	9.48 ± 0.50	8.93 ± 0.08	18.62 ± 0.54

ND: No determined; CN: Gentamicin; C-POJ (untreated purple onion juice); P-POJ (thermal pasteurized purple onion juice juice); TS-POJ (thermosonication-treated purple onion juice). Results are presented mean ± standard deviation (n = 3).

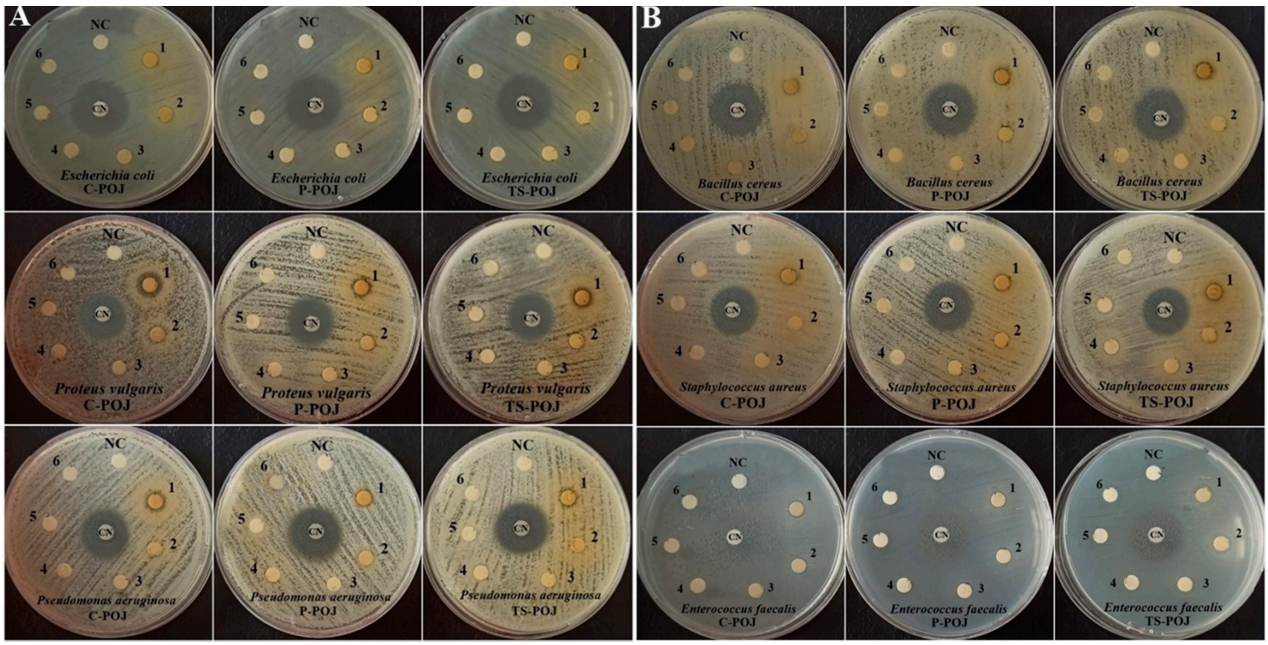

Figure 3. Antibacterial activity of six different concentrations of C-POJ, P-POJ, and TS-POJ (%100–3.125) against Gram-negative (**A**) and Gram-positive (**B**) bacteria. CN: Gentamicin, NC: Negative control.

No other study investigating the antibacterial activity of POJ produced by thermosonication and thermal pasteurization methods has been reported in the literature to the authors' knowledge. Two Gram-positive bacteria (Staphylococcus aureus ATCC 25923 and *Listeria monocytogenes* ATCC 19115) and three Gram-negative bacteria (*Salmonella* Typhimurium ATCC 14028, *Escherichia coli* ATCC 8739 and *Campylobacter jejuni* ATCC 33291) strains were used in a study investigating the antibacterial activity of onion essential oil by the disc diffusion method [34]. It was found that onions have strong antibacterial effects on Gram-positive and Gram-negative bacteria. In another study, the antibacterial activity of polysaccharide fractions of onion against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhimurium bacteria* was investigated using the agar diffusion method.

All fractions showed antibacterial activity, but the largest inhibition zone diameter was detected for the dilute alkaline soluble solid (DASS) fraction [35]. In conclusion, onions have a significant antimicrobial effect when considering the results of this study and similar studies in the literature.

3.4. ACE Inhibitory and Antidiabetic Activities

ACE inhibitory activity and antidiabetic activity of C-POJ, P-POJ, and TS-POJ samples are shown in Figure 4. As a result of the optimization, a attempt was made to explain the effect of increasing the bioactive components of purple onion juice on ACE, α-glucosidase and α-amylase activities. ACE inhibitory activities of C-POJ, P-POJ, and TS-POJ samples were determined as 23.48%, 21.57%, and 26.08%, respectively. The TS-POJ sample retained greater ACE inhibition ($p > 0.05$) compared to the C-POJ sample (Figure 4A).

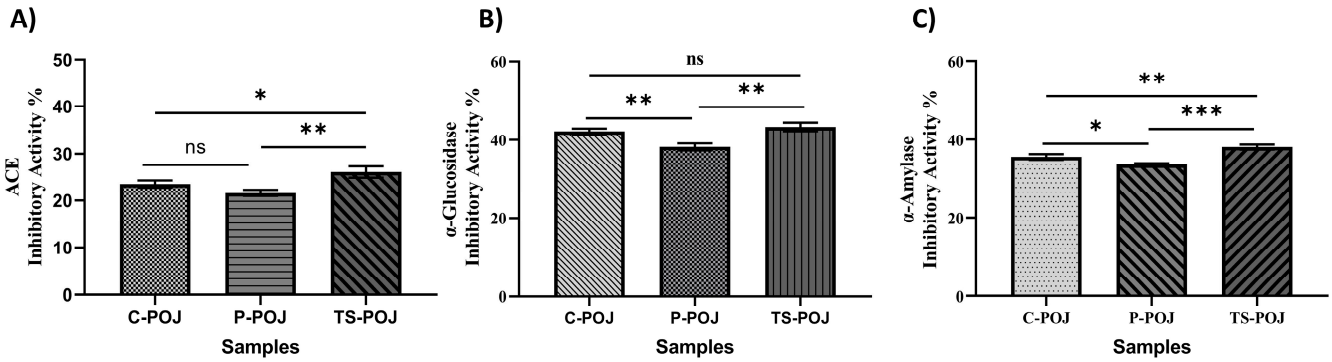

Figure 4. Antihypertensive (**A**) and antidiabetic inhibitory activities (**B**,**C**) of untreated purple onion juice (C-POJ), thermosonication-treated purple onion juice (TS-POJ), and thermal pasteurized purple onion juice (P-POJ). The letters at the top of the bars indicate statistically significant differences (ns: not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$), (n = 3 ± SD).

When Figure 4B was examined, α-glucosidase inhibitions of C-POJ, P-POJ, and TS-POJ samples were determined to be 42.16%, 38.22%, and 43.29%, respectively. The highest α-glucosidase inhibition was detected for the TS-POJ sample with 43.29%, while the P-POJ sample showed the lowest activity with 38.22%. When Figure 4C was examined, α-amylase inhibitions of C-POJ, P-POJ, and TS-POJ samples were determined to be 35.51%, 33.51%, and 38.08%, respectively. When α-amylase inhibition was examined in Figure 4, the TS-POJ (38.08%) sample provided more protection than the P-POJ (33.51%) sample. As a result of thermal pasteurization and thermosonication processes applied to purple onion juice, an increase in the antihypertensive and antidiabetic activities was detected. It was reported that the increase in bioactive compounds that occur with the effect of cavitation in the ultrasound treatment of organic cherry laurel (Prunus laurocerasus) vinegar can increase antidiabetic and antihypertensive effects [13]. Similar results indicated that the increase in the content of bioactive compounds with thermosonication treatment applied to freshly squeezed pomegranate juice may explain the increase in antihypertensive and antidiabetic effects [7].

3.5. Ascorbic Acid Activities

When examining Figure 5, the amounts of ascorbic acid in samples C-POJ, P-POJ, and TS-POJ were determined as 12.25 ± 0.52 mg/100 mL, 9.52 ± 0.36 mg/100 mL, and 10.49 ± 0.20 mg/100 mL, respectively. The highest amount of ascorbic acid, 12.25 ± 0.52 mg/100 mL, was detected in the C-POJ sample, while the P-POJ sample exhibited the lowest activity with 9.52 ± 0.36 mg/100 mL. A decrease in the ascorbic acid content was observed after thermal pasteurization and thermosonication applications compared to the C-POJ sample, but the thermosonication process better preserved the amount of ascorbic acid. The TS-POJ process (10.49 ± 0.20 mg/100 mL) resulted in a smaller reduction in ascorbic acid content compared to the P-POJ process (9.52 ± 0.36 mg/100 mL).

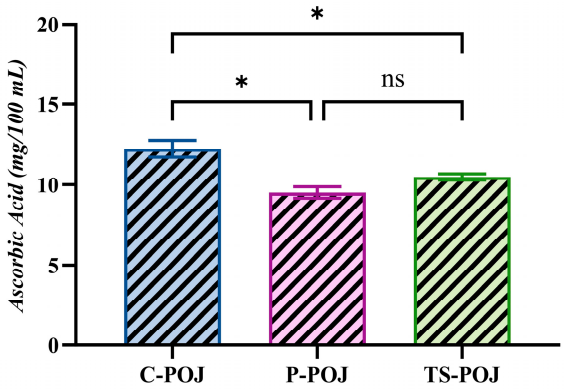

Figure 5. Ascorbic acid results of untreated purple onion juice (C-POJ), thermal pasteurized purple onion juice (P-POJ), and thermo-sonication-treated purple onion juice (TS-POJ). Letters atop bars indicate statistically significant differences (ns: not significant; * $p < 0.05$), (n = 3 ± SD).

In contrast to the results in our study, Çöl et al. (2023) found in their research that there was no statistically significant decrease in the ascorbic acid value in sour cherry juice compared to the control sample after the application of thermal pasteurization and thermosonication processes [8]. In a study conducted by Putsakum et al. (2023), the effect of thermosonication on antioxidant compounds in blackberry juice was investigated. Similar to our study, this research observed that, compared to pasteurization, thermosonication better preserved the ascorbic acid content of blackberry juice [36]. Another study showed that the ascorbic acid content of a tropical fruit juice blend was significantly reduced after pasteurization [37]. In the study conducted by Sahu et al. (2023), it was observed that thermosonication treatments applied to Nagpur mandarin juice showed better ascorbic acid and carotenoid content compared to fruit juice samples pasteurized thermally [38]. Microshock waves and cavitation were thought to be effective.

4. Conclusions

Purple onion juice is an important vegetable juice. It is known for its bioactive compounds, nutritional value, and flavor profile. The bioactive components in the purple onion juice were enriched by the thermosonication process. RSM and ANN models used for enrichment were found to perform better, while ANN was more predictive. Purple onion juice samples showed good antidiabetic and antihypertensive effects. Thermosonication increased antidiabetic and antihypertensive effects. The current study findings also revealed that purple onion juice exhibited a marked antiproliferative effect against A549, HeLa, and Caco-2 cells in a dose-dependent manner, regardless of the preparation method, thermal pasteurization or thermosonication processes. Furthermore, the antibacterial effect was present with pure concentrations of purple onion juice samples prepared using C-POJ, TS-POJ, and P-POJ. Therefore, thermosonication can be considered as a potential processing technique to improve purple onion juice quality. Future in vivo studies will be guided by the results of these studies.

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