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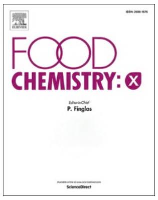

ThermoMicrowave-sonication improves the stability and digestive bioaccessibility of phenolic compounds in parsley juice☆

Seydi Yıkmış [a,*], Nazan Tokatlı Demirok [b], Aylin Duman Altan [c], Ishak Pacal [d,e], Melikenur Türkol [b], Nazlı Tokatlı [f], Nurettin Pacal [g], Gholamreza Abdi [h,*], Rana Muhammad Aadil [i,*]

[a] Department of Food Technology, Tekirdag Namık Kemal University, 59830 Tekirdag, Turkiye
[b] Department of Nutrition and Dietetics, Tekirdağ Namık Kemal University, Tekirdag 59030, Turkiye
[c] Department of Industrial Engineering, Tekirdag Namık Kemal University, 59860 Tekirdağ, Turkiye
[d] Department of Computer Engineering, Faculty of Engineering, Igdir University, 76000 Igdir, Turkiye
[e] Department of Electronics and Information Technologies, Faculty of Architecture and Engineering, Nakhchivan State University, Nakhchivan AZ 7012, Azerbaijan
[f] Department of Computer Engineering, Faculty of Engineering and Natural Sciences, Istanbul Health and Technology University 34421 Istanbul, Turkiye
[g] Igdır University Research Laboratory Application and Research Centre, 76000 Igdır, Turkiye
[h] Department of Biotechnology, Persian Gulf Research Institute, Persian Gulf University, Bushehr 75169, Iran
[i] National Institute of Food Science and Technology, University of Agriculture, Faisalabad 38000, Pakistan

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ABSTRACT

These are indications of the effects of ThermoMicrowave Sonication (TMS) on the bioactive compounds of parsley (*Petroselinum crispum*) juice and their bioaccessibility during in vitro digestion. Total phenolic content (TPC), iron-reducing antioxidant power (FRAP), chlorophyll, and ascorbic acid levels were measured in TMS-treated and pasteurized samples. TMS minimized the loss of heat-sensitive proteins and significantly increased the phenolic content and antioxidant structure ($p < 0.05$). By following simulated oral, gastric, and intestinal digestion, TPC, chlorophyll, and FRAP levels were better in TMS samples than in controls or pasteurized samples. The highest recoverable levels were observed in the intestinal phase, highlighting the role of TMS in supporting functional quality after digestion. Prediction models using linear regression and LASSO showed strong accuracy ($R^2 > 0.99$) for antioxidant capacity. Overall, TMS offers a promising, environmentally friendly, and industrially applicable tool for preserving and ensuring bioaccessibility of bioactive images in parsley juice and valuable information for functional electrical development.

Chemical compounds: Gallic acid (PubChem CID:370); flovone (PubChem CID: 10680); vanillic acid (PubChem CID: 8468); rutin (PubChem CID: 5280805); naringin (PubChem CID: 442428); p- coumaric acid (PubChem CID: 637542); o- coumaric acid (PubChem CID: 637540); quercetin (PubChem CD: 5280459); alizarin (PubChem CD: 6293).

1. Introduction

Parsley [*Petroselinum crispum* (Mill.) Fuss] is a medicinal and aromatic plant from the Apiaceae family, originating from the Mediterranean and widely cultivated worldwide (Subaş et al., 2024). Parsley is a fragrant biennial plant with characteristic features such as hairy, finely divided leaves, umbrella-like flower clusters, schizocarpous fruits, and an unbranched taproot (Punoševac et al., 2021). The strong antioxidant capacity of parsley is due to the abundant flavonoids (apigenin, luteolin, quercetin) and phenolic acids (chlorogenic acid, ferulic acid) present in its leaves and roots. In addition, essential nutrients such as vitamin C, iron, and potassium further enhance its antioxidant profile (Ahmed et al., 2025). The bioactive compounds in parsley make it more than just a food and make the plant highly valuable in both functional food production and the nutraceutical industry. Studies have shown that parsley possesses potent antioxidant and anti-inflammatory properties. This potent effect is due to the presence of flavonoids and phenolic compounds such as apigenin, rutin, quercetin, and luteolin (Chudzińska-

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* Corresponding authors.

E-mail addresses: syikmis@nku.edu.tr (S. Yıkmış), abdi@pgu.ac.ir (G. Abdi), muhammad.aadil@uaf.edu.pk (R.M. Aadil).

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Skorupinska et al., 2025;ननizioł-Łukaszewska et al., 2025). Parsley, a valuable ingredient in functional beverage formulations, possesses a comprehensive bioactive profile with potent antioxidant and anti-inflammatory properties. However, the thermal instability of these beneficial components poses a risk of oxidative and thermal degradation during conventional processing.

Innovative technologies are replacing traditional heat treatments to enhance functional properties and preserve bioactive components. High temperatures can negatively impact both the nutritional value and sensory attributes of heat-sensitive foods (Srivastava & Sit, 2025). To improve properties such as taste and aroma in fruit juices without causing nutrient loss, scientists have investigated innovative processing methods that do not use heat (Maia et al., 2025). Microwave technology is an environmentally friendly and effective method that uses electromagnetic energy and direct heating to vaporize volatile compounds in plant samples (Velisdeh et al., 2024). Microwaves provide uniform and rapid heat treatment through internal heating, while ultrasound disrupts cell walls and rearranges dissolved components through cavitation. These methods help preserve heat-sensitive compounds while keeping energy usage low, thus better preserving antioxidant and nutritional properties (Alam et al., 2023; de Linhares et al., 2020). Thermomicrowave-sonication (TMS), a hybrid technology combining microwave and ultrasound, offers an important alternative in this context. However, a review of existing scientific publications revealed no studies applying this combined method to parsley juice. The literature lacks original studies investigating the effects of TMS on phenolic compounds, bioactive capacity, or the potential bioaccessibility of parsley juice.

The effects of TMS application on parsley juice were investigated through a detailed analysis of experimental data using advanced statistical methods. Linear regression and LASSO (Least Absolute Shrinkage and Selection Operator) techniques were used in the study to identify relationships between variables and increase the predictive power of the model. Linear regression allows for future predictions based on observations by modeling the relationship between a continuous independent variable and a continuous dependent variable (Lee Johnson et al., 2012). LASSO regression is used to identify important variables and determine their corresponding regression coefficients, which helps minimize estimation errors in the linear regression model (Bautista-Romero et al., 2025).

In this study, we examined how TMS treatment affects fresh parsley juice, focusing on total phenolic compound content, antioxidant capacity, and potential bioaccessibility. This innovative treatment, which involves non-thermal or minimal heat treatment, demonstrates the potential to improve the functional properties of plant-based extracts, addressing a significant gap in the literature. The study also aims to provide a scientific basis for natural and sustainable food processing techniques.

2. Materials and methods

2.1. Preparation of parsley juice

Parsley (*Petroselinum crispum*) samples were obtained from local agricultural producers in the Tekirdağ region of Türkiye and stored under controlled conditions at +4 °C to ensure biochemical stability during the preliminary analysis period. Stems and mature tissues were removed during sample preparation. Mechanical homogenization was performed using a Waring brand commercial blender (Model HGB2WTS3), ensuring particle size homogenization. The suspension was passed through Whatman No. 1 filter paper to remove cellulosic residues, and then its macromolecular distribution was standardized with a vortex mixer (2000 rpm, 1 min) (Fig. S1). Untreated parsley juice was designated as the control group (C-PJ).

2.2. Thermal pasteurization and thermomicrowave-sonication treatments

Pasteurization was carried out by heating the parsley juice using a water bath (WB-22-P, Germany) at 85 °C for 2 min and then rapidly cooling to 4 °C. As a result, pasteurized parsley juice was obtained (P-PJ). The parsley juice was thermosonicated using a WB-22-P (Germany) water bath at 60 %, 80 % and 100 % ultrasonic amplitudes, at 40 °C, 50 °C and 60 °C for 8, 12 and 16 min. Then, microwave irradiation was applied for 200, 450 and 700 W power levels using a Samsung ME711K (Kuala Lumpur, Malaysia) microwave device (TMS-PJ) for 20, 25 and 30 s.

2.3. Modeling procedure for linear regression and LASSO

Within the experimental design, TMS parameters were first optimized using LR (Linear Regression) and LASSO methods, with the FRAP value used as the response. Parsley juice processed under ideal TMS conditions resulting from the optimization was compared with conventional thermal pasteurized and control samples. This approach allows for systematic evaluation of the effects of TMS application on antioxidant capacity and comparison of the performance of different processing methods. The multiple regression method aims to minimize the sum of squared errors ($RSS = \sum\left[Y - B_0 - \sum\left(B_i{*}X_i\right]^2\right)$ when predicting a dependent variable with more than one independent variable. The least squares method has limitations, especially when a large number of variables are involved or when multicollinearity can lead to overfitting by increasing the model variance (Tibshirani, 1996). To address this obstacle and to enable easier comparisons between different analyses, an additional model using LASSO for each output variable was developed. LASSO, a special form of linear regression introduced by Tibshirani (1996), has become a standard tool in machine learning. This method allows for both parameter minimization and variable selection by applying a penalty term ($L1 = \lambda\sum|B_i|$) to the regression coefficients (Melkumova & Shatskikh, 2017). The residual minimizing model is mathematically expressed as the sum of squares, provided that the sum of the absolute values of the coefficients is less than a tuning parameter λ (Andriopoulos & Kornaros, 2023):

$$\min\left(\frac{1}{2n}\sum\left[Y - B_0 - \sum\left(B_i{*}X_i\right]^2 + \lambda\sum|B_i|\right)\right. \tag{1}$$

n: number of observations.
B_0 = intercept.
B_i = regression coeffients.
X_i = independent variables.
λ = regulation parameter.
RSS = residual sum of squares.

In this study, various preparation and optimisation steps were undertaken to improve the performance of the Linear Regression and LASSO models. The data were first normalised using StandardScaler, and then second-order polynomial terms were added to capture complex relationships between variables. The optimal parameters for the LASSO model were determined using GridSearchCV.

R^2, MAPE, and RMSE performance parameters were used to assess the predictive accuracy of Lasso and Linear Regression and to compare their performance. Parsley juice prepared using the determined optimum TMS parameters was used for comparative analysis. This approach allows systematic evaluation of the effects of TMS application on antioxidant capacity and other quality parameters.

2.4. Determination of bioactive compounds

Total phenolic content was determined using the Folin-Ciocalteu method, and results were expressed as milligrams of gallic acid equivalent per liter (Singleton & Rossi, 1965). Iscorbic acid in parsley juice was measured using 2,6-dichloroindophenol titration following the method described by Ordóñez-Santos et al. (2017) (Ordóñez-Santos et al., 2017).

Results are presented as ascorbic acid in milligrams per 100 mL of parsley juice. Chlorophyll content was measured using the spectrophotometric method described by Hiscox & Israelstam (1979). (Hiscox & Israelstam, 2011).

In the methodological approach, 3 mL of parsley juice sample was extracted with an equal volume of 80 % (*v*/v) acetone. The resulting crude mixture was filtered three times through Whatman filter paper to obtain a clear filtrate. Chlorophyll content was determined by spectrophotometrically measuring the absorbance of this clear liquid at 645 nm and 663 nm. The FRAP (Iron Reducing Antioxidant Power) assay protocol was used to determine the total antioxidant capacity. This protocol is based on the reduction of Fe^{3+} ions to Fe^{2+} by antioxidants, producing a colored species that absorbs at 593 nm. The absorbance of this species was recorded, and the results were reported as mmol Trolox equivalents per liter using a calibration curve prepared from Trolox, a synthetic antioxidant (Thaipong et al., 2006).

2.5. *Analysis of phenolic compounds*

Quantitative analysis of phenolic compounds was performed using an Agilent 1260 Infinity high-performance liquid chromatography (HPLC) system equipped with a diode array detector (DAD). An ACE Generix C18 column (Advanced Chromatography Technologies Ltd., Aberdeen, Scotland) with dimensions of 250 × 4.6 mm and a particle diameter of 5 μm was used in the analyses. During the analysis, the column temperature was maintained at 30 °C, and the mobile phase flow rate was set at 0.80 mL/min. The mobile phase consisted of two solvents: phase A was pure water containing 0.1 % phosphoric acid, and phase B was acetonitrile. The gradient program for the separation was as follows: 17 % B at the beginning; 15 % B at 7 min; 20 % B at 20 min; 24 % B at 25 min; 30 % B at 28 min; 40 % B at 30 min; 50 % B at 32 min; 70 % B at 36 min; and 17 % B at 40 min. For the determination of phenolic compounds, a sample volume of 10 μL was injected for each run. Detection was carried out at wavelengths of 280, 320, and 360 nm. The analyzed phenolic compounds included gallic acid, ferulic acid, hydroxybenzoic acid, vanillic acid, p-coumaric acid, o-coumaric acid, gentisic acid, quercetin, alizarin, rutin, resveratrol, naringin, flavonoids, trans-cinnamic acid, and neohesperidin. Calibration curves were established for each compound in the concentration range of 2.5–250 mg/L, and validation results demonstrated high measurement accuracy and reliability, with R^2 values above 0.99 for all compounds. This approach ensured reliable quantitative determination of phenolic compounds and increased the analytical accuracy of the study. Results were expressed in micrograms per millilitre (μg/mL) (Portu et al., 2017).

2.6. *In vitro simulated gastrointestinal digestion analysis*

In vitro digestion of parsley juice samples was performed by adapting the method of (Minekus et al., 2014) with additional procedural details. Samples were first subjected to the oral stage, where α-amylase (75 U/mL) was added and gently mixed for 2 min at 37 °C and pH 7.0. This was followed by the gastric stage with 2 h incubation at pH 3.0 and 37 °C using pepsin. Pancreatin and fresh bile were used for the small intestinal stage, and the process was completed at pH 7.0 and 37 °C for 2 h. After the gastric and intestinal stages, samples were homogenized and centrifuged, and total chlorophyll, TPC, and FRAP values were determined. All experiments were repeated with three parallel samples, and control samples without enzymes were used to monitor possible effects of pH and incubation conditions. The percent recovery (bioaccessibility) of each compound after in vitro digestion was calculated according to the following Eq. 2.

$$\text{Recovery}\ (\%) = (C_a/C_u)x100 \qquad (2)$$

C_a = concentration of the compound after digestion (bioaccessible fraction, mg/100 mL or μg/mL).

C_u = concentration of the compound before digestion (undigested sample).

2.7. *Statistical analysis*

The data obtained in the study were analyzed using different statistical software. Statistical evaluations were made using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Three-dimensional surface response graphs were plotted using Sigma Plot 12.0 (Systat Software Inc., San Jose, CA, USA) and linear regression and Lasso analyses were performed using Minitab 18.1.1 (Minitab Inc., USA) software. Experimental data are presented as the mean ± standard deviation (SD) of three parallel samples for each treatment. One-Way Analysis of Variance (ANOVA), Tukey's Multiple Comparison Test and Independent Samples *t*-test were used to determine the differences between groups. The level of significance was accepted as $p < 0.05$.

3. Results and discussion

3.1. *Optimization of FRAP*

According to the modeling performed using LASSO and linear regression methods to estimate the FRAP value (Table 1), the optimum points for all input variables were obtained at the same values. These optimum points are 16 min, 70 %, 60 °C, 20 s, 200 W for Time (min), Amplitude (%), Temperature (°C), Time (s), and Power (W), respectively. The estimation results of the FRAP value based on the optimum points were determined to be 13.38 by the LASSO method and 13.45 by the linear regression method.

As illustrated in Fig. S3, it is clear that when the temperature is high, the FRAP value increases, and conversely, when the power is high, the FRAP value decreases. Additionally, it can be inferred that the Time (min) variable is almost ineffective in the model.

The most accurate prediction of the FRAP value (with 3.1 %) at the optimum points belongs to linear regression. Notably, linear regression performed better in FRAP estimation based on optimum points. This result suggests that using multiple methods for analysis would be more helpful in comparing the results (Table S1).

The regression lines, prepared using the data set based on the actual and predicted values of FRAP presented in Table S1, are shown in Figs. 1 and 2. As illustrated in Figs. 1 and 2, the actual vs predicted values of FRAP are very close to each other in both methods, indicating the robustness and predictive accuracy of the models.

3.2. *LASSO and linear regression model comparison*

LASSO and Linear Regression models were applied to optimize the TMS parameters, using the FRAP value as the response variable. However, since the primary objective of the study was to evaluate the effects of TMS application on antioxidant capacity, the comparison of both models is summarized only to demonstrate the reliability of the optimization results.

Statistical indicators, including R^2 (ranging from 0.993 to 0.994), RMSE (ranging from 0.115 to 0.129), and MAPE (ranging from 0.052 to 0.057), demonstrated robust prediction capabilities and tolerable prediction errors with low bias for all constructed models. Even with a slight difference, the predictive power of the LASSO regression method is slightly higher (Table 2).

The findings demonstrate a compelling performance compared to studies in the literature that use similar methods. Especially when compared to the prediction accuracy in the study of (Kottaridi et al., 2023), the high R^2 and low error metrics obtained in this study indicate that the models used are statistically strong and applicable in practice. In their study comparing machine learning and deep learning models for the evaluation of grape quality, (Swe & Noguchi, 2024) found that linear

Table 1
Optimization results in Linear regression and Lasso regression.

Model	Output	Time (min)	Amplitude (%)	Temperature ©	Time (s)	Power (W)	Predicted Output
Linear Regression	FRAP	16	70	60	20	200	13.45
Lasso Regression	FRAP	16	70	60	20	200	13.38

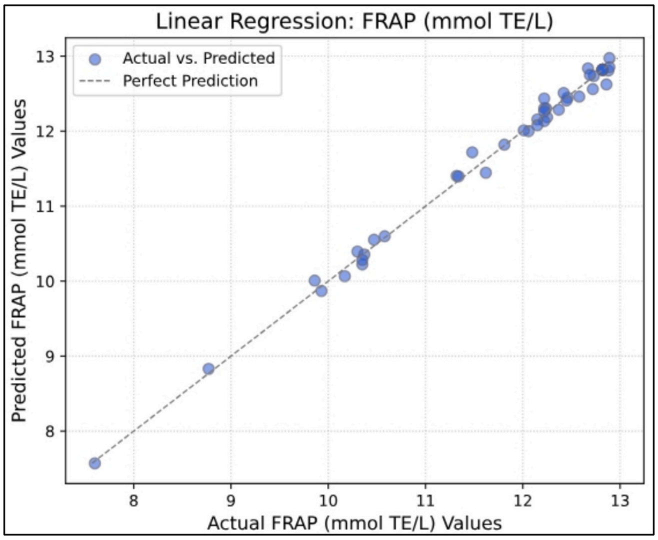

Fig. 1. Actual vs. predicted values of FRAP based on Linear regression.

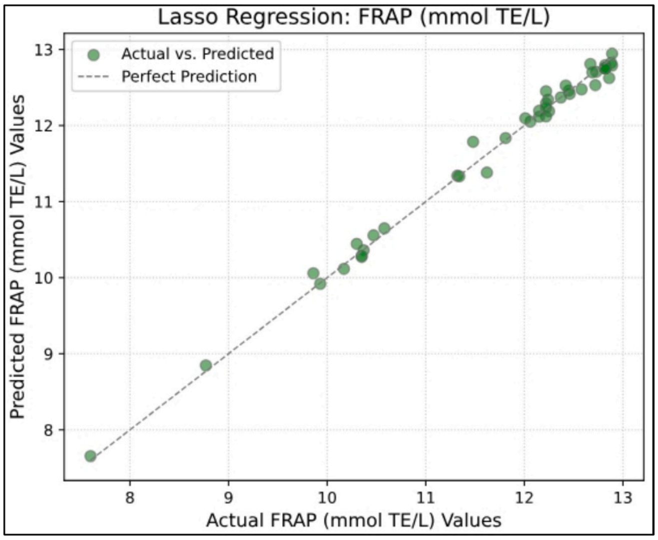

Fig. 2. Actual vs. predicted values of FRAP based on LASSO regression.

Table 2
LASSO regression and Linear regression comparison.

Parameters	FRAP (mmol TE/L)	
	LASSO regression	Linear regression
R^2	0.994	0.993
RMSE	0.115	0.129
MAPE	0.052	0.057

Ridge and LASSO regression models exhibited superior performance with relatively higher accuracies. However, the limited increase in prediction errors may indicate the need for more precise tuning of model parameters or evaluation of hybrid modeling approaches in the future. Overall, the findings provide a significant contribution to the literature both in terms of methodology and prediction performance.

R^2: R-squared; RMSE: Root Mean Square Error; MAPE: Mean Absolute Percentage Error; FRAP: ferric-reducing antioxidant power; LASSO: Least absolute shrinkage and selection operator

3.3. *Bioactive compounds*

Parsley is a source of bioactive compounds that stand out with its high phenolic compound, ascorbic acid, flavonoid, and carotenoid content (Ertik et al., 2023). The bioactive compound profile of parsley juice showed significant changes as a result of the different treatments applied. While the ascorbic acid content was determined as 123.77 mg/100 mL in the control group, this value was largely maintained at 120.58 mg/100 mL with TMS (thermo-microwave-sonication) application. In contrast, pasteurization reduced the ascorbic acid content to 110.14 mg/100 mL. When evaluated statistically, no significant difference was found between the TMS and control groups ($p > 0.05$), while a significant difference was found between the TMS and pasteurized groups ($p < 0.05$). These results show that ascorbic acid is a temperature-sensitive compound and that the TMS treatment causes less thermal degradation than conventional heat treatment. Dulger Altıner et al. (2024) evaluated the effects of thermosonication (TS) on ascorbic acid and total chlorophyll content in parsley juice. The ascorbic acid content was 142.83 mg/100 g in the control group and 139.23 mg/100 g after TS application, with no statistically significant difference ($p > 0.05$). A significant decrease in ascorbic acid content was observed in pasteurized samples ($p < 0.05$). These results show that the TS process effectively preserves heat-sensitive components such as ascorbic acid. Total chlorophyll values were also examined within the scope of the study, and it was determined that the TS process positively protected or increased these components. These findings show that TS causes less thermal degradation than conventional heat treatments and is an effective alternative in preserving bioactive components (Dulger Altıner et al., 2024). Significant differences were also observed between the groups in terms of total chlorophyll content. Total chlorophyll level reached the highest level in the TMS group with 7.65 mg/100 mL, which was 10.4 % higher than the control sample (6.93 mg/100 mL) and 33.7 % higher than the pasteurized sample (5.72 mg/100 mL). Statistical analysis showed significant differences between the pasteurized group and the control and TMS groups ($p < 0.05$). These findings suggest that high temperatures negatively affect the stability of chlorophyll, while TMS application is more effective in preserving this pigment. The correlation heat map (Fig. S4) shows positive correlations among chlorophyll, TPC, and FRAP, supporting the idea that pigment stability is related to overall functional capacity. Similarly, Yıkmış et al. (2025) evaluated the effects of thermosonication on the total chlorophyll and ascorbic acid contents in broccoli juice. Their results indicate that thermosonication is effective in increasing the total chlorophyll content. When certain parameters were optimized during thermosonication, significant increases in total chlorophyll content were observed (Yıkmış et al., 2025).

When TPC was evaluated, the highest value of 142.01 mg GAE/100 mL was obtained with TMS application. This value was 5.8 % higher than the control group (134.26 mg GAE/100 mL) and 12 % higher than the pasteurized group (126.77 mg GAE/100 mL). Analysis of variance revealed that these differences were statistically significant ($p < 0.05$). Correlation analysis also determined a strong positive relationship between TPC and FRAP ($r = 0.93$, $p < 0.01$). This shows that the TMS treatment improved the total bioactive capacity in terms of both quantity and functional activity by increasing the extraction of phenolic compounds. In a study conducted by Hussain et al. (2024), sonication and microwave treatments were applied to sugarcane juice together with mint, and significant increases were obtained in TPC values. In particular, the highest TPC values were obtained with a 15-min ultrasonication process. This increase was reported to be related to ultrasonication's disruption of cell walls and the release of bound phenolic compounds. In contrast, microwave treatment enhances the release of phenolic compounds by degrading macromolecules at low temperatures (Hussain et al., 2024). Similarly, another study investigated the synergistic effect of microwave heating and thermosonication on the physicochemical and nutritional quality of a mixture of melon and sugarcane juice. It was reported that TPC values increased due to these two treatments, and the increase was statistically significant. The highest TPC values were obtained with 15 min of sonication and 120 s of microwave treatment (Fatima et al., 2023). In another study investigating a combined microwave-ultrasonic treatment process to obtain anthocyanin-rich mulberry juice, the effects of this combination on the TPC and antioxidant capacity of black mulberry juice were evaluated. Under optimal conditions (microwave time of 46 s and addition of 273 mg/kg citric acid), the TPC value was determined to be 4.24 mg GAE/mL, which represented a 96.3 % increase compared to the control group. It was found that low-frequency ultrasonic treatment (25 kHz) could significantly reduce the loss of total phenolic and anthocyanin monomers (Xu et al., 2022). In a study conducted by Qiu et al. (2024), the effects of thermosonication on blackcurrant juice were evaluated in terms of antioxidant capacity, total phenolic content, anthocyanin content, and ascorbic acid content (Qiu et al., 2024). The results show that thermosonication effectively increased the levels of these bioactive components. Notably, significant increases in TPC values were observed with the application of thermosonication (Xu et al., 2022). Fig. 3 shows the results of TPC, ascorbic acid, total chlorophyll and FRAP tests in parsley samples.

Regarding FRAP value, the TMS process gave the highest result among the groups (13.42 mmol TE/L). This value was 6.4 % higher than that of the control group (12.61 mmol TE/L) and 25.3 % higher than that of the pasteurized group (10.71 mmol TE/L) ($p < 0.05$).

The differences between the groups were statistically significant ($p < 0.05$). Heat map analysis found positive and significant correlations between FRAP and TPC ($r = 0.93$) and between FRAP and total chlorophyll ($r = 0.85$). These data reveal the decisive effect of phenolic compounds and pigments on antioxidant capacity. In general, TMS treatment is effective in improving the functional quality of parsley juice by preserving bioactive compounds and increasing antioxidant capacity. In one study, the effects of a high-intensity thermosonication treatment on the bioactive compounds, rheological properties, microbiological properties, and enzymatic properties in spinach juice were evaluated. The results show that thermosonication effectively increased TPC and antioxidant capacity (FRAP). In particular, significant increases in FRAP values were observed with thermosonication application. These increases were associated with thermosonication disrupting cell walls and releasing bound phenolic compounds (Manzoor et al., 2021).

3.4. Phenolic compounds profile

Polyphenolic compounds are one of the largest and most effective substances in plants. They are secondary plant metabolites (Poureini et al., 2020). HPLC performed qualitative analysis of phenolic compounds in the samples, and the results are presented in Fig. S2 together

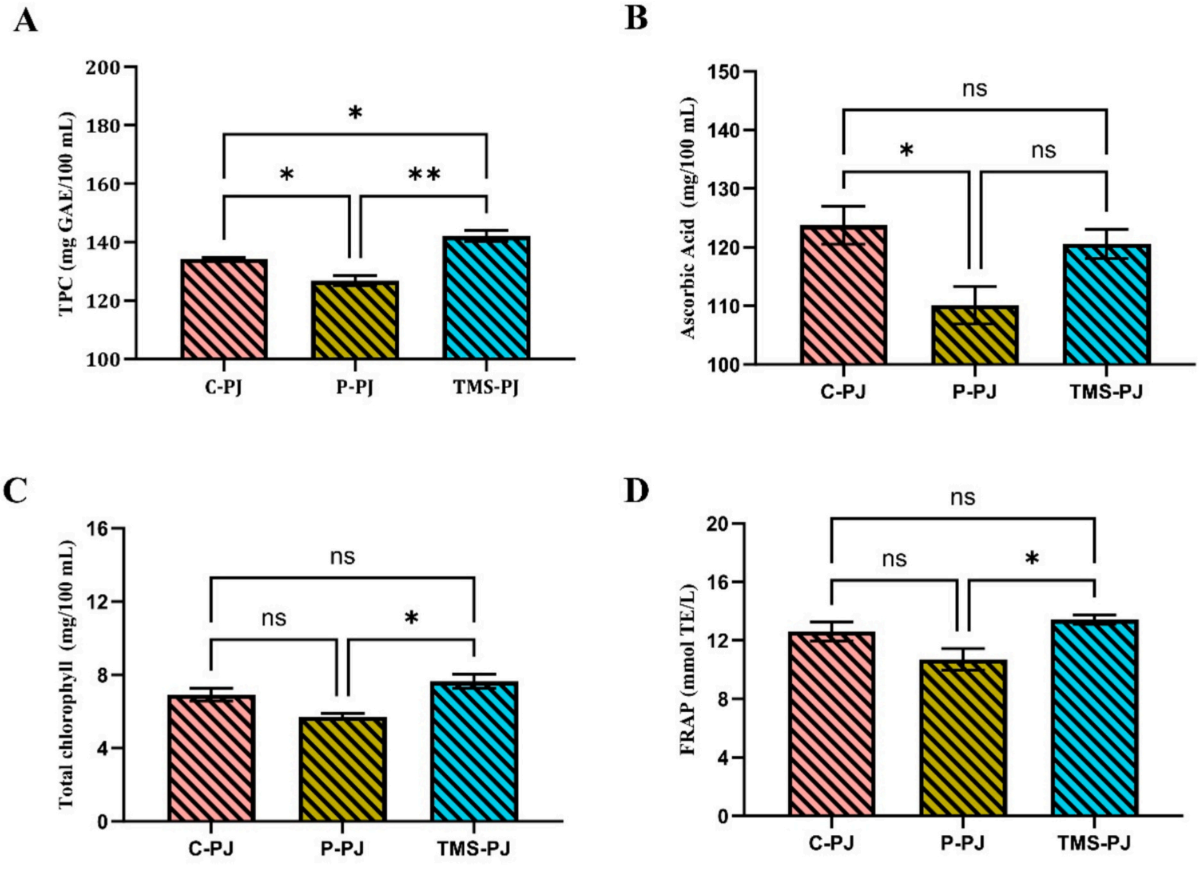

Fig. 3. Levels of parsley juice samples subjected to control (C-PJ), pasteurization (P-PJ), and thermo-microwave sonication (TMS-PJ) treatments: (A) TPC (mg GAE/100 mL), (B) Ascorbic acid (mg/100 mL), (C) Total chlorophyll (mg/100 mL), and (D) Antioxidant capacity – FRAP (mmol TE/L). Different symbols within the same parameter indicate statistically significant differences between groups ($p < 0.05$). *p < 0.05; **$p < 0.01$; ns: not significant. ($n = 3$).

with the corresponding chromatogram. In the study, TMS application caused significant increases in most of TPC, resulting in significant increases in both TPC levels and antioxidant capacity (FRAP) values. Total phenolic content (82.00 ± 0.63 μg/mL) in the TMS-PJ group was found to be 30 % and 45 % higher than that in the control (63.14 ± 2.44 μg/mL) and pasteurized (56.35 ± 0.79 μg/mL) groups, respectively ($p < 0.05$). Gallic acid, one of the main components of this increase, reached 51.22 μg/mL in TMS application and statistically significantly exceeded the levels in the control and pasteurized groups (Table S2). Similarly, Dülger Altıner et al. (2024) found a significant increase in the amount of gallic acid in parsley juice as a result of thermosonication compared to the pasteurized and control groups (Dulger Altıner et al., 2024). This indicates that gallic acid is more effectively released from the cell matrix by the synergy of heat, ultrasound, and microwave, thus increasing its bioaccessibility. Similarly, naringin content showed the highest value in the TMS sample (20.48 ± 1.39 μg/mL) and was notable with a 103 % increase compared to the pasteurized group ($p < 0.05$). Similarly, Ioannou et al. (2020) reported a 20 % decrease in naringin after two hours of heat treatment at 130 °C (Ioannou et al., 2020).

TMS treatment had a profound effect on the total content and phenolic compound profile. Increases in compounds with high antioxidant capacity, such as quercetin, vanillic acid, and p-coumaric acid, led to significant improvements in antioxidant activities, which were primarily supported by FRAP assays. Positive and statistically significant correlations ($r > 0.80$, $p < 0.01$) were observed between these compounds and FRAP in the correlation heatmap. For example, quercetin level increased to 1.11 μg/mL in the TMS group and was significantly different ($p < 0.05$) from both control (1.06 μg/mL) and pasteurized (0.70 μg/mL) samples. Similarly, Velisdeh et al. (2024) found that combined microwave and ultrasound treatment significantly increased quercetin levels (Velisdeh et al., 2024). These findings suggest that TMS technology has a protective or liberating effect on flavonoid-type phenolics. In contrast to our study, Alifakı et al. (2022) observed significant improvements in routine correlations following the application of ultrasound and microwave technology (Alifakı et al., 2022). This may be due to differences in data reproduction and processing during the application processes. In addition, neohesperidin was detected only in the treated samples and reached 2.00 μg/mL in the TMS group, which was 31 % higher than the value in the pasteurized sample (1.52 μg/mL). The absence of this compound in the control group suggests that TMS is superior in liberating new or bound phenolics. In contrast, alizarin showed a significant decrease with TMS application (0.51 ± 0.02 μg/mL), with the highest level measured in the pasteurized sample (3.43 ± 0.28 μg/mL). This result suggests that TMS application may cause degradation or structural transformation of certain phenolic compounds. Additionally, the flavone compound was not detected with TMS treatment. These decreases indicate that TMS does not preserve all phenolic structures and that the effects of heat, microwaves, or ultrasound may degrade some compounds. However, when evaluated in terms of overall effect, it is clear that TMS treatment caused a positive transformation in the phenolic profile in terms of both quantity and function. The high correlation between TPC and FRAP in the TMS group ($r > 0.90$, $p < 0.01$) supports the idea that the increase in phenolic compounds is directly related to the increase in functional capacity (Fig. S4). Overall, parsley juices processed with TMS technology were richer in phenolic compounds and more functional. Pearson correlation analysis revealed strong positive correlations between TPC and FRAP and total chlorophyll levels, demonstrating a multidimensional effect of TMS. The bioaccessibility of phenolic compounds increased in samples processed with this technology, thereby directly increasing antioxidant capacity. These data demonstrate that the TMS process is a modern and effective technology that improves both preservative and functional effects compared to conventional pasteurization. This supports its industrial applicability, particularly in functional beverage development studies.

3.5. PCA and cluster analysis of processing effects on bioactives

To assess the bioactive compound contents of pre-digestion samples and the relationships between these compounds, principal component analysis (PCA) and cluster analysis were applied to data from the treated and control groups. These analyses were conducted to reveal the effects of different processing conditions on the bioactive compound profile at the pre-digestion level. The results illustrate the similarities and differences between pre-digestion samples and explain the impact of processing methods on bioactive compound distribution.

PCA results (Fig. 4-A) demonstrated that 100 % of the total variance was represented, with the first component (PC1) accounting for 69 % and the second component (PC2) for 31 %. This high explanatory power shows that the processing methods (control, pasteurization, and TMS) caused significant differences in the bioactive compounds. TMS-PJ samples clustered in the PCA plane with +PC1 loadings in the same direction as TPC (142.01 mg GAE/100 mL), FRAP (13.42 mmol TE/L), total chlorophyll (7.65 mg/100 mL), naringin (20.48 μg/mL), quercetin (1.11 μg/mL), and gallic acid (51.22 μg/mL). This indicates that TMS helps enhance antioxidant capacity by aiding the release of phenolic compounds. The control group (C-PJ) was more associated with hydroxybenzoic acid (1.60 μg/mL) and p-coumaric acid (1.88 μg/mL), showing a positive loading on PC2. The pasteurized group (P-PJ) was separated in the negative directions of PC1 and PC2 in the PCA plane, especially with alizarin (3.43 μg/mL) and flavone (1.30 μg/mL). These negative loadings indicate that pasteurization reduces overall functional capacity by degrading certain phenolic compounds ($p < 0.05$).

The hierarchical clustering dendrogram (Fig. 4-B) supports the findings from PCA. Two main clusters were observed: the first cluster included ascorbic acid (120.58 mg/100 mL, TMS) and TPC, and it was determined that these two parameters changed in parallel during digestion and processing. The second cluster encompassed FRAP, chlorophyll, and flavonoids (e.g., quercetin, vanillic acid, naringin), and it was observed that these parameters synergistically contributed to functional capacity. The proximity of the TMS samples to these clusters suggests that TMS enhanced positive interactions among bioactive compounds. Conversely, the distant position of the P-PJ samples indicates that heat treatment weakened the correlations between phenolic profiles and antioxidant capacity, resulting in negative interactions in certain compounds. These results imply that TMS not only preserved individual compounds but also supported the relationships and functional integrity among bioactive compounds.

3.6. Bioaccessibility of chlorophyll, total phenolic compounds, and FRAP activity following ın vitro simulated gastrointestinal digestion

Significant differences in total chlorophyll levels were observed in three differently treated parsley juice samples (C-PJ: control, P-PJ: thermal pasteurized, TMS-PJ: thermo-microwave-sonication) during the simulated digestion process (Table 3). Total chlorophyll levels decreased significantly in each phase of digestion, with the loss being most pronounced during the intestinal phase. These results show that TMS technology is more successful in maintaining chlorophyll stability under digestion conditions. Additionally, when the recovery rates were analyzed, it was found that the TMS-PJ samples had the highest recovery rate, at 31.30 ± 0.39 %. This rate was lower in the P-PJ and C-PJ samples, at 28.56 ± 0.07 % and 28.34 ± 0.35 %, respectively. The degradation of chlorophylls into pheophytins and pheophorbides during digestion has a significant influence on their bioaccessibility. Previous studies have reported that, following in vitro digestion, total chlorophyll profiles typically consist of approximately 90 % pheophytins (Viera et al., 2022).It was also reported that conventional heat treatments severely degraded these pigments in kale samples, while high-pressure treatment preserved total chlorophyll and carotenoids more effectively (Schmidt et al., 2021). On the other hand, applying microencapsulation techniques has been shown to improve chlorophyll stability and

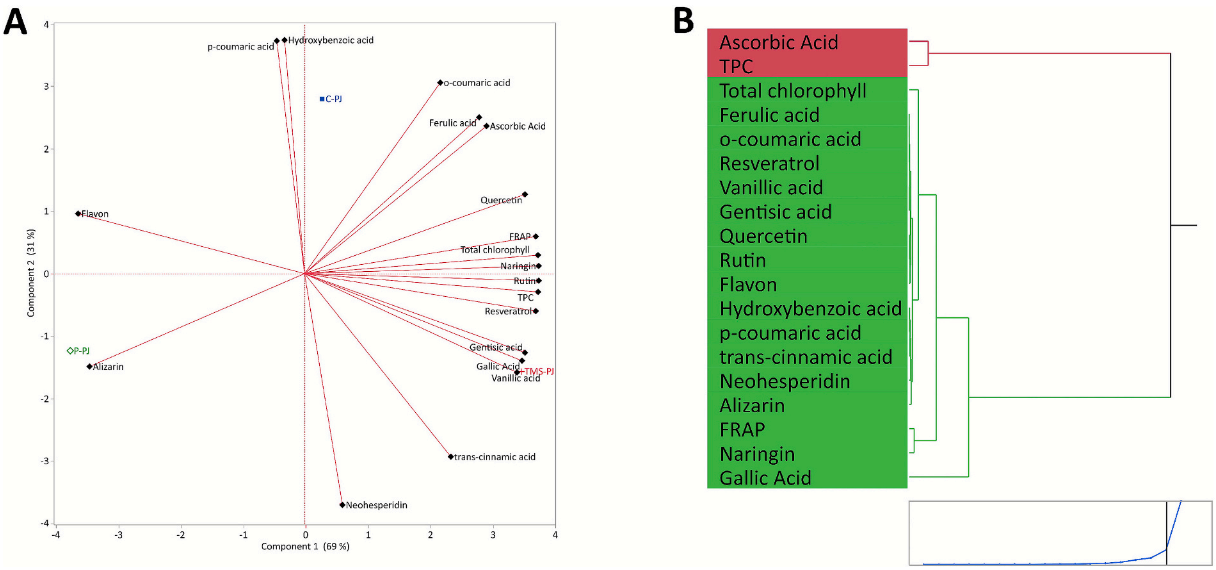

Fig. 4. (A) Principal component analysis (PCA) biplot showing the distribution of control (C-PJ), pasteurized (P-PJ), and ThermoMicrowave-Sonication-treated (TMS-PJ) parsley juices about phenolic compounds, ascorbic acid, total phenolic content (TPC), total chlorophyll, and antioxidant capacity (FRAP). (B) Hierarchical clustering dendrogram illustrating the correlation structure and similarity patterns among the analyzed bioactive compounds and antioxidant parameters.

bioaccessibility (Agarry et al., 2023), suggesting that the protective mechanisms afforded by TMS may confer similar advantages by mitigating extensive degradation.

Table 3
Total chlorophyll, TPC, and FRAP during simulated digestion in C-PJ, P-PJ, and TMS-PJ samples.

Phases	Samples	Total chlorophyll (mg/100 mL)	TPC (mg GAE/ 100 mL)	FRAP (mmol TE/L)
Undigested	C-PJ	6.93 ± 0.13^{b}	134.16 ± 1.56^{b}	12.42 ± 0.58^{b}
	P-PJ	5.66 ± 0.21^{a}	127.16 ± 2.73^{a}	10.70 ± 0.36^{a}
	TMS-PJ	7.50 ± 0.33^{b}	140.77 ± 1.12^{c}	13.88 ± 0.67^{c}
Oral digestion	C-PJ	5.89 ± 0.11^{b}	114.93 ± 1.52^{b}	10.31 ± 0.48^{a}
	P-PJ	4.81 ± 0.17^{a}	109.36 ± 2.34^{a}	8.88 ± 0.30^{a}
	TMS-PJ	6.52 ± 0.29^{c}	121.99 ± 0.28^{c}	11.90 ± 0.81^{b}
Gastric digestion	C-PJ	4.19 ± 0.14^{b}	88.49 ± 1.17^{b}	7.73 ± 0.36^{b}
	P-PJ	3.29 ± 0.16^{a}	84.21 ± 1.81^{a}	6.66 ± 0.22^{a}
	TMS-PJ	4.60 ± 0.23^{b}	93.27 ± 1.05^{c}	8.92 ± 0.61^{c}
Intestinal digestion	C-PJ	1.96 ± 0.06^{b}	52.87 ± 2.10^{b}	4.64 ± 0.21^{b}
	P-PJ	1.62 ± 0.06^{a}	44.31 ± 1.86^{a}	3.71 ± 0.10^{a}
	TMS-PJ	2.35 ± 0.12^{c}	58.18 ± 1.68^{c}	5.58 ± 0.21^{c}
Recovery %	C-PJ	28.34 ± 0.35^{a}	39.41 ± 1.74^{b}	37.36 ± 0.02^{b}
	P-PJ	28.56 ± 0.07^{a}	34.85 ± 1.58^{a}	34.64 ± 0.32^{a}
	TMS-PJ	31.30 ± 0.39^{b}	41.34 ± 1.50^{b}	40.26 ± 1.32^{c}

C-PJ: Control parsley juice; P-PJ: Thermal pasteurized parsley juice; TMS-PJ: Thermo microwave-sonication parsley juice; TPC: Total phenolic content; FRAP: Ferric reducing antioxidant power; mg GAE: milligram gallic acid equivalent; mmol TE: millimol trolox equivalent; different letters within the same parameter indicate statistically significant differences ($p < 0.05$).

TPC was considered an essential indicator of bioactive components and was measured at each stage of digestion. TMS-PJ samples had the highest TPC value, 140.77 ± 1.12 mg GAE/100 mL, at the beginning, followed by C-PJ (134.16 ± 1.56 mg GAE/100 mL) and P-PJ (127.16 ± 2.73 mg GAE/100 mL). Although there were significant decreases in TPC values in each phase of digestion, the TMS-PJ group maintained significantly higher values in both oral and gastric phases ($p < 0.05$). In the small intestine phase, the TMS-PJ sample presented the highest value, at 58.18 ± 1.68 mg GAE/100 mL, while the values for the C-PJ and P-PJ samples were 52.87 ± 2.10 and 44.31 ± 1.86 mg GAE/100 mL, respectively. A similar trend was observed regarding TPC recovery rate; the TMS-PJ sample provided a statistically significantly higher recovery with 41.34 ± 1.50 %. The bioaccessibility of phenolic compounds is highly variable, influenced by the food matrix, specific digestive conditions, and processing history (Chen et al., 2014). The TMS application is also practical in terms of TPC values. The TMS-treated parsley juice (TMS-PJ) group exhibited significantly greater preservation of TPC during the oral and gastric phases of digestion. During the intestinal phase, the TMS-PJ sample showed the highest total phenolic content, measuring 58.18 ± 1.68 mg GAE/100 mL. These results are consistent with the findings of (Reche et al., 2023), who reported a 45 % increase in the post-digestive release of phenolic compounds in chili samples pretreated with high-power ultrasound in the gas phase. The study also demonstrated that ultrasonic treatments enhanced the bioaccessibility of phenolics.

When FRAP values reflecting the antioxidant capacity were analyzed, it was similarly determined that TMS-PJ samples were superior in all digestion phases. Although the antioxidant capacity, which was measured as 13.88 ± 0.67 mmol TE/L in the TMS-PJ sample at the initial phase, decreased to 5.58 ± 0.21 mmol TE/L at the end of the intestinal phase, this decrease was less than that of the other groups and the value was statistically significantly higher ($p < 0.05$). For example, FRAP values of C-PJ and P-PJ samples in the same phase were 4.64 ± 0.21 and 3.71 ± 0.10 mmol TE/L, respectively. In terms of FRAP recovery rate, the TMS-PJ group exhibited the highest values, at 40.26 ± 1.32 %, which was significantly higher than those of the C-PJ (37.36 ± 0.02 %) and P-PJ (34.64 ± 0.32 %) samples ($p < 0.05$). The FRAP values mirrored the trends observed for total chlorophyll and TPC, with TMS-PJ samples consistently exhibiting superior antioxidant capacity throughout the simulated digestion (Table 3). It has been stated that the amount of phenolic acid tripled after digestion in blueberry samples subjected to the thermosonication process, and this group maintained the highest antioxidant capacity (Wu et al., 2021). Similar studies have shown that under simulated digestion conditions, the concentration of phenolic compounds can increase in certain samples due to their release by digestive enzymes (Nayak et al., 2022). Furthermore, phenolics extracted using innovative techniques such as microwave-assisted extraction have been reported to remain stable during digestion (Jaouhari et al., 2025). These findings highlight the strong correlation between antioxidant activity and TPC in such processed samples. The combined thermal and non-thermal effects of TMS (e.g., cavitation, mechanical stress) may offer a more effective strategy for maintaining or enhancing the release and stability of antioxidant compounds during digestion compared to conventional thermal processing.

The comprehensive analysis of the data (Table 3, Fig. S4) indicates that conventional thermal pasteurization (P-PJ) hurts the stability of total chlorophyll, TPC, and antioxidant capacity in parsley juice during simulated digestion. In stark contrast, the TMS treatment consistently resulted in the highest retention and recovery of these bioactive compounds. It has been demonstrated that ultrasound and thermosonication can enhance the retention of chlorophyll, ascorbic acid, phenolic compounds, and mineral content in products like broccoli juice, offering a sustainable improvement over traditional methods (Yıkmış et al., 2025). Similarly, thermosonication has been reported to increase TPC and antioxidant capacity in blackcurrant juice (Qiu et al., 2024) and affect the bioaccessibility of bioactive compounds in pomegranate juice during in vitro digestion, where processing plays a crucial role in retention (Yıkmış et al., 2022).

Overall, conventional P-PJ was observed to negatively impact total chlorophyll, phenolic compounds, and antioxidant capacity. On the other hand, TMS treatment yielded the highest values for all post-digestion bioaccessibility parameters. This suggests that the use of more innovative, multi-technology-based (combined) treatments instead of traditional heat treatments can significantly enhance the functionality of bioactive compounds. The results suggest that TMS technology can be evaluated for its ability to preserve nutrients and serve as a platform for enhancing bioaccessibility in the development of functional foods.

4. Conclusions

In this study, the effects of TMS technology on the bioactive compound content, chlorophyll level, ascorbic acid content, and antioxidant capacity of parsley juice were comprehensively evaluated. The results showed that TMS application preserved bioactive compounds more effectively than conventional pasteurization and could increase the amounts of some compounds; in particular, statistically significant increases were observed in TPC, FRAP, and chlorophyll levels with TMS application ($p < 0.05$). An in vitro simulated gastrointestinal digestion model demonstrated that TMS treatment increased post-digestive bioaccessibility. The effects of the application parameters were modeled using linear and LASSO regression analyses, and both methods were shown to provide high accuracy in FRAP predictions. These findings demonstrate that TMS technology is an effective method for preserving bioactive compounds, improving functional properties, and increasing post-digestive bioaccessibility. It stands out as a sustainable, innovative, and industrially viable alternative for the production of functional beverages.

CRediT authorship contribution statement

Seydi Yıkmış: Writing – original draft, Validation, Software, Resources. **Nazan Tokatlı Demirok:** Writing – review & editing, Writing – original draft, Conceptualization. **Aylin Duman Altan:** Writing – review & editing, Writing – original draft, Visualization. **Ishak Pacal:** Methodology, Investigation. **Melikenur Türkol:** Software, Investigation. **Nazlı Tokatlı:** Funding acquisition, Formal analysis, Data curation, Conceptualization. **Nurettin Pacal:** Investigation, Funding acquisition, Formal analysis. **Gholamreza Abdi:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Data curation, Conceptualization. **Rana Muhammad Aadil:** Writing – review & editing, Writing – original draft, Software, Investigation.

Ethical approval

No human or animal studies are included in this article.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.103406.

Data availability

Data will be made available on request.

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