

Optimization of bioactive components of ultrasound treated white grape juice

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Abstract

Grapes are among the most commonly produced fruits worldwide, thanks to their ease of cultivation in terms of climate and soil requirements. Owing to their abundant vitamins, bioactive compounds and minerals incorporating them into one's diet is advised for promoting human health. The bioactive components of ultrasound-treated white grape juice were optimised by response surface methodology (RSM). In addition, untreated white grape juice (C-WGJ), ultrasound treated white grape juice (U-WGJ) and thermally pasteurized white grape juice (P-WGJ) samples were compared for total phenolics compound (mg GAE/L), total flavonoids (mg CE/L) and DPPH (% Inhibition) parameters. The scientific investigation employed RSM, a widely favored approach. During the study, ultrasound process parameters such as amplitude (40, 50, 60, 70 and 80%) and duration (2, 4, 6, 8 and 10 minutes) were manipulated. The responses to the process application were assessed through analyses of total phenolic content (TPC), total flavonoid content (TFC) and DPPH (1,1-Diphenyl-2-Picryl Hydrazyl). The model achieved commendably high R² values following optimization through RSM. One-way ANOVA for DPPH value, TFC and TPC of samples, both amplitude and duration exhibited statistically significant effects ($p < 0.001$). The effectiveness of ultrasound treatment in increasing bioactive components in untreated white grape juice is higher than thermal pasteurization treatment. Through the optimization process, the best bioactive values for white grape juice were achieved, measuring 42.3 mg CE/L for TFC, 55.5% inhibition for DPPH and 440.3 mg GAE/L for TPC. Ultrasound applied to white grape juice proved to be more effective in preserving and enhancing bioactive compounds than thermal pasteurization. Consequently, the findings suggest the need for further investigations to assess the impacts of both thermal pasteurization and ultrasound technology on additional quality parameters of white grape juice.

Keywords: Bioactive substances, RSM, Ultrasound

INTRODUCTION

Globally, grapes stand out as the most extensively grown fruits, appreciated for their adaptability to various climates and soil conditions. Their categorization as "red" or "white" hinges on the presence of anthocyanins in their outer skin (Georgiev et al., 2014). Cultivated across continents, grapes thrive in regions boasting adequately, warm, rainy and dry summers, coupled with relatively mild winters. Turkey, given its climatic conditions, emerges as a bountiful reservoir of grapes (Vivier & Pretorius, 2000). In their skin, white grapes harbor flavonoids (isorhamnetin-3-O-glucoside, quercetin 3,4'-O-glucoside, kaempferol-3-O-glucuronide, quercetin 3-O-glucoside, quercetin 3-O-glucuronide, and

kaempferol-3-O-glucoside) and flavanols (proanthocyanidins, catechin, epicatechin) with a particular emphasis on the richness of these compounds (Sabra et al., 2021). Economically attainable and delectable, grapes are advocated as a healthful dietary choice for humans, given their abundance in bioactive compounds, minerals, and vitamins (Fernandes et al., 2017). Anticarcinogenic, anti-obesity, antiviral, and antioxidant effects are manifested by bioactive components (Zhou et al., 2022).

The objective of food processing is to generate products that are more convenient, attractive, value-enhanced, and possess an extended shelf life. The conventional approach to food processing primarily employs heat-based methods to manage the development of foodborne pathogens and curtail microbial growth. However, when applied to foods sensitive to heat, these procedures may induce alterations in color, taste, and consistency (Singla & Sit, 2021). In lieu of thermal methods, cutting-edge technology, specifically ultrasound-assisted processing has been devised. This approach is recognized as a health-conscious, environmentally friendly, secure and non-thermal method for physical processing (Zhang et al., 2023). Numerous scholars have determined that employing non-thermal methods, such as ultrasound treatment, results in minimal diminishment of nutritional value and quality across a range of food products. These include tangerine juice (Tokatlı Demirok & Yıkımsı, 2022), cranberry vinegar (Erdal et al., 2022), pineapple juice (Hoque et al., 2022) including physicochemical properties, antioxidant activities, and microbial inactivation, were studied. Pineapple juice was sonicated at 100 W and 140 W (for 5, 10 and 15 min, each) and apricot juice (Sattar et al., 2020) microwaved for 1.5 min at 850 W of power and sonicated for 90 min at 20 kHz of frequency were selected to keep in storage for up to 30 days in refrigerator to examine the changes happened to their physicochemical characteristics and functional components. It was observed that the pH and the cloud values of all processed juice samples reduces with the storage time, whereas, the total soluble solids almost remain consistent particularly in microwave and ultrasound treated samples. While storage period causes the decrement in total phenolic content (TPC, rosehip nectar (Atalar et al., 2020), black grape juice (Yıkımsı et al., 2023) and guava juice (Kalsi et al., 2023).

RSM is a statistical approach that demands a reduced investment of time and effort. It has been consistently and effectively applied for the continuous optimization of various contents (Ruby-Figueroa et al., 2023). RSM is a popular technique for multivariate statistical methods. RSM is a persistent system combination based on the fit to the stored polynomial model, which should result in the storage of all data for the purpose of fitting the expanding model for predictions (Ghorbannezhad et al., 2016). Following a comprehensive review of the existing literature, no investigations were identified pertaining to the optimization of bioactive components in white grape juice through RSM. The main objective of the study was to optimise the bioactive components (TPC, TFC, DPPH) of ultrasonically treated white grape juice using RSM and to produce white grape juice rich in bioactive components. At the same time, untreated white grape juice (C-WGJ), ultrasonically treated white grape juice (U-WGJ) and thermally pasteurised white grape juice (P-WGJ) samples will be compared in terms of TPC, TFC, DPPH parameters.

MATERIALS AND METHODS

Preparation of white grape juice samples

In the research, fresh white grapes were carefully harvested from Tekirdag, Turkiye. The juice was crafted by utilizing a blender (Waring Commercial Blender Model HGB2WTS3, USA) to crush the grapes. Following filtration through a double-layer cheesecloth, the resultant white grape juice underwent sterilization and was then bottled in 200 mL airtight containers. The untreated white grape juice served as the control (C-WGJ). Another batch of white grape juice underwent pasteurization in a water bath at 90 °C for 30 seconds, with subsequent rapid cooling to 20 °C (P-WGJ). Additionally, a 200 W ultrasonic processor (Hielscher Ultrasonics Model UP200St, Berlin, Germany) operating at a frequency of 26 kHz was employed to process another 200 mL of white grape juice (U-WGJ). Maintaining temperature control throughout the ultrasound procedure was achieved through the utilization of an ice bath. Subsequently, all specimens were preserved at -18 °C until the analytical stage.

Experimental structure

Leveraging RSM, the fine-tuning of bioactive constituents in the white grape variant has been conducted using ultrasound technology, known for its superior attributes over thermal pasteurization. The ultrasound procedure encompassed variables including duration minutes (2, 4, 6, 8, 10) and amplitude (40%, 50%, 60%, 70%, 80%). Analyses of TPC, TFC and DPPH were executed as responses to the application of the process. A Central Composite Design was opted for and a two-factor, five-level experimental arrangement was formulated (Table 1). A total of 13 experimental setups were devised for this investigation. Adequacy values of the model were assessed through lack-of-fit tests, ANOVA results, and the consideration of R^2 and adjusted $-R^2$ coefficients. The independent variables were defined within the temporal (X_1) and amplitude (X_2) ranges.

Table 1. The dependent and independent parameters of the RSM assay and the results of bioactive compounds

Sample ^a	Encoded Independent Variables		Dependent Variables					
	Time (X ₁)	Amplitude (X ₂)	Response 1		Response 2		Response 3	
			Total Phenolics Compound (mg GAE/L)		Total flavonoids (mg CE/L)		DPPH (% Inhibition)	
			Experimental data	RSM predicted	Experimental data	RSM predicted	Experimental data	RSM predicted
1	8	50	440.48±1.05	440.06	41.77±1.73	41.72	54.60±0.86	54.48
2	8	70	439.83±0.44	439.29	42.35±0.31	42.34	55.50±0.52	55.46
3	6	60	438.16±1.86	438.26	41.78±0.79	41.68	54.60±1.95	54.70
4	2	60	427.44±1.50	426.87	41.13±0.91	41.08	53.10±0.48	53.10
5	6	60	438.16±1.41	438.26	41.68±0.11	41.68	54.80±1.13	54.70
6	6	60	438.16±0.23	438.26	41.66±0.62	41.68	54.72±2.12	54.70
7	6	60	438.27±0.71	438.26	41.66±0.72	41.68	54.72±0.4	54.74
8	10	60	438.45±0.64	438.99	42.50±0.38	42.53	54.64±0.72	54.70
9	4	70	437.75±1.85	438.34	41.59±0.68	41.67	53.76±1.07	53.81
10	6	80	441.41±1.37	441.47	42.22±0.79	42.19	55.04±0.88	55.03
11	4	50	428.17±0.85	428.89	40.90±0.35	40.94	54.48±0.54	54.51
12	6	60	438.58±2.23	438.26	41.66±1.41	41.68	54.80±0.25	54.74
13	6	40	432.89±1.90	432.79	40.82±1.09	40.84	54.74±0.44	54.76
C-WGJ			425.76±1.07		40.18±0.24		52.18±0.65	
P-WGJ			410.44±0.79		38.55±1.22		49.65±0.57	
U-WGJ- Multiple Response Prediction			440.30		42.30		55.50	
Experimental values			435.26±1.78		41.15±0.92		53.44±0.28	
% Difference			1.14		2.72		3.71	

RSM: Response surface methodology C-WGJ: white grape juice P-WGJ: thermal pasteurized white grape juice U-WGJ: ultrasound-treated white grape juice TPC: total phenolic content; TFC: total flavonoid content; DPPH: radical scavenging activity; GAE: gallic acid equivalent; CE: equivalent of catechins

Determination of total phenolic components

Folin-Ciocalteu method developed by Singleton and Rossi (1965) was used for TPC detection (Singleton & Rossi, 1965). 0.5 mL samples of fruit juice, suitably diluted with distilled water, were withdrawn and 2.5 mL of 0.2 N Folin-Ciocalteu reagent was introduced. Following a 3-minute interval, 2 mL of 7.5% (w/v) Na₂CO₃ solution was incorporated. The vials were then placed in a dimly lit environment at room temperature for 30 minutes. Subsequently, the absorbance value was gauged at a wavelength of 760 nm utilizing a UV-VIS spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Melbourne, Australia). The TPC was quantified in milligrams of gallic acid equivalents per liter (mg GAE/L). The analyses were conducted in triplicate.

Determination of total flavonoid components

TFC was calculated colorimetrically using a UV spectrophotometer (Spectrum Instrument, SP-UV/VIS-300SRB, Australia) (Zhishen et al., 1999). A certain amount (1 mL) of the sample was added to a 10 mL tube containing 4 mL of double-distilled water. Then, 0.3 mL of 5% NaNO₂ was added to the tube, and after 5 minutes, 0.3 mL of AlCl₃ (10%) was added. At the 6th minute, 2 mL of NaOH (1 M) was added, and the total volume was made up to 10 mL with double-distilled water. The tubes were left in a dark environment at room temperature for 30 minutes. Subsequently, the absorbance value was measured at a wavelength of 510 nm using a UV-VIS spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Melbourne, Australia). The total flavonoid content was expressed as milligrams of catechin equivalents (CE) per liter. The experiments for total flavonoid analysis were conducted in triplicate.

DPPH free-radical scavenging activity

Evaluation of antioxidant activity was carried out using DPPH radical (Grajeda-Iglesias et al., 2016). For 1 mL of white grape juice, 1 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (0.2 mM in methanol) was introduced, followed by incubation in the absence of light at room temperature (25 ± 1 °C) for 30 minutes. The analyses were conducted in triplicate. Alterations in absorbance were measured at 517 nm using a spectrophotometer (SP-UV/VIS-300SRB, Melbourne, Australia). The outcomes were quantified as % inhibition.

Statistical analysis

All values were obtained in triplicate and expressed as mean \pm standard deviation (SD). The RSM was performed using the Minitab software package (version 19. Minitab Software); the RSM plots were developed using the SigmaPlot 12.0 software package (Systat Software. Inc.).

RESULTS AND DISCUSSION

Optimization of bioactive components

Non-thermal pasteurization is a technique devised to mitigate alterations in the nutritional and sensory attributes of food. Serving as a substitute for thermal methodologies, this approach is pioneering and ecologically sustainable (Bhargava et al., 2021; Fan et al., 2021; Valiati et al., 2022). This non-thermal technology offers several advantages such as rapid processes, enhanced process efficiency, elimination of process steps, better quality product and retention of product characteristics (texture, nutrition value, organoleptic properties). White grape juice is a beverage renowned for its elevated antioxidant activity, attributed to the presence of flavonoids and flavanols in its composition. The bioactive constituents in foods can be impacted by elevated temperatures. Due to their high temperatures, thermal treatments can negatively affect the sensory appeal and nutritional quality of food, making them vulnerable to adverse effects. Consequently, aside from preservation, there arises a necessity for the augmentation of bioactive components in white grape juice through non-thermal methods. Another objective of this study is to amplify the bioactive constituents of white grape juice via ultrasound treatment. The optimization outcomes for TPC (mg GAE/L) TFC (mg CE/L) and DPPH (%inhibition) content of white grape juice are delineated in Table 2.

Table 2. ANOVA results of bioactive components (TPC, TFC and DPPH)

Source	DF	TPC (mg GAE/L)		TFC (mg CE/L)		DPPH (% Inhibition)	
		F-Value	P-Value	F-Value	P-Value	F-Value	P-Value
Model	5	157.08	0.000	171.49	0.000	152.25	0.000
Linear	2	280.06	0.000	416.05	0.000	192.24	0.000
X ₁	1	371.19	0.000	447.65	0.000	373.36	0.000
X ₂	1	188.94	0.000	384.45	0.000	11.12	0.013
Square	2	68.72	0.000	12.23	0.005	122.19	0.000
X ₁ *X ₁	1	136.66	0.000	6.03	0.044	197.72	0.000
X ₂ *X ₂	1	6.10	0.043	11.70	0.011	6.54	0.038
2-Way Interaction	1	87.81	0.000	0.88	0.379	132.39	0.000
X ₁ *X ₂	1	87.81	0.000	0.88	0.379	132.39	0.000
Error	7						
Lack-of-Fit	3	19.61	0.007	1.90	0.271	1.43	0.358
Pure Error	4						
Total	12						
R ²		99.12%		99.19%		99.09%	
Adj. R ²		98.49%		98.61%		98.44%	
Pred. R ²		91.53%		94.85%		94.64%	

X₁: time; X₂: amplitude; DF: degrees of freedom; R²—coefficient of determination; DPPH: radical scavenging activity; TFC: total flavonoid content; TPC: total phenolic content; GAE: gallic acid equivalent; CE: equivalent of catechins; p <0.05, significant differences; p <0.01, very significant differences.

Following the optimization, Formula (1) outlines the second-degree modeling equation for the TPC value in white grape juice, expressed in milligrams of gallic acid equivalents per liter (mg GAE/L).

$$\text{TPC mg GAE/L} = 348.03 + 13.180X_1 + 1.321X_2 - 0.3330X_1X_1 - 0.00281X_2X_2 - 0.1278X_1X_2 \quad (1)$$

Table 2 provides the R² values, analysis of variance (ANOVA), lack-of-fit assessment, and regression coefficients. White grapes, an integral part of the Mediterranean diet, are recognized for their health advantages and substantial levels of flavonols and phenolic acids (Montalbano et al., 2021).

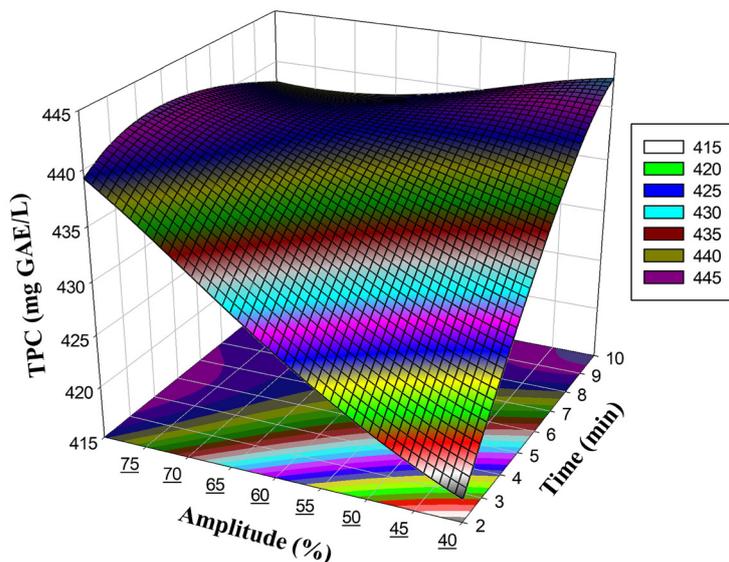


Figure 1. Response surface plots (3D) of TPC as functions of significant interaction factors

Our investigation revealed that ultrasound treatment led to an elevation in the TPC value of white grape juice. A 3.42% enhancement was noted in comparison to the C-WGJ sample. Three-dimensional RSM graphs were generated to pinpoint the optimal values of independent variables for bioactive components (DPPH, TPC, and TFC). The impact of ultrasound on TPC was elucidated through the response surface graph illustrated in Figure 1. Upon examining the impacts of time and amplitude, a consistent augmentation in the TPC (mg GAE/L) amount was observed. The RSM modeling level exhibited a robust fit with an R^2 value of 99.12% (Table 2). Both the one-way and two-way effects of the model were statistically significant ($p < 0.05$). Subsequent to the optimization process, the TPC was ascertained to be 440.3 mg GAE/L (Table 1). In a study conducted by Kalsi et al. (2022) investigating the influence of ultrasound on the quality of guava juice, akin to our findings, a noteworthy surge in the TPC (3.50–4.35%) was observed post-ultrasound treatment. ($p < 0.05$) (Kalsi et al., 2023). The thermosonication process treated to black grape juice (*Vitis vinifera L.*) (Yıkımsı et al., 2023) has increased the TPC value of grape juice. The findings in these studies are consistent with our study, indicating that thermal pasteurization processes treated similarly to grape juices further reduce the TPC. In other studies comparing ultrasound treatment and thermal pasteurization on fruit juices, it was observed that ultrasound treatment not only protected but also enriched the bioactive components of fruit juices. The fact that the thermal pasteurization process causes a decrease in the amounts of all bioactive components can be explained as the high temperature applied damages the structure of the compounds (Kahraman & Feng, 2021; Tokatlı Demirok & Yıkımsı, 2022).

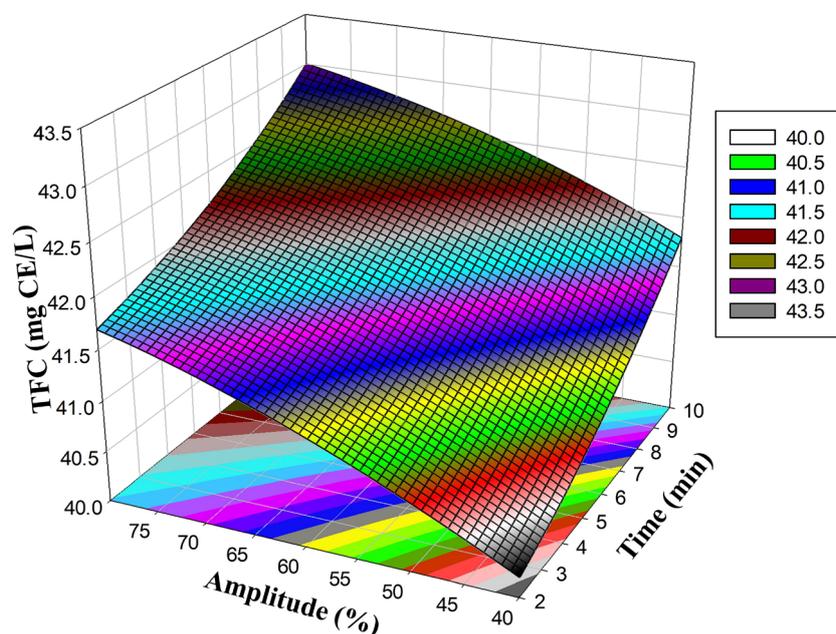


Figure 2. Response surface plots (3D) of TFC as functions of significant interaction factors

Following the RSM, the R^2 value for TFC demonstrated a substantial correlation at 99.19% (Table 2). The second-degree polynomial equation for the enhancement of white grape juice is delineated in Formula (2).

$$\text{TFC}(\text{mg CE/L}) = 36.800 + 0.1744X_1 + 0.0933X_2 + 0.00765X_1X_1 - 0.000426X_2X_2 - 0.00140X_1X_2 \quad (2)$$

In the one-way (ANOVA) for TFC, both duration and amplitude exhibited statistical significance ($p < 0.001$), whereas in the two-way interaction, TFC did not reach statistical significance ($p > 0.05$). Subsequent to optimization, the TFC value was established at 42.30 mg CE/L. Post-pasteurization, TFC was measured at 38.55 ± 1.22 mg CE/L. Following ultrasound treatment, a 5.27% rise was noted in comparison to the C-WGJ sample. In the P-WGJ sample, a 4% decrease was observed in contrast to the C-WGJ sample (Table 1). The ultrasound process exhibited superior preservation of the TFC in white grape juice when compared to thermal pasteurization. The impact of ultrasound on TFC is elucidated through the response surface graph depicted in Figure 2. Both the time and amplitude effects were observed to enhance the quantity of TFC (mg CE/L). In a study conducted by Manzoor et al. (2023), analogous to our investigation, an elevation in TFC and TPC values was noted following sonication in the assessment of the impact of sonication on the general characteristics of the quality of the tomato juice (Faisal Manzoor et al., 2023). Tokatlı Demirok et al. (2023) documented that the application of ultrasound elevated the TFC in conventional apple vinegar enriched with horsetail-fortified (Tokatlı Demirok et al., 2023). In their investigation, Yıkmiş et al. (2021) observed that the application of ultrasound enhanced the bioactive constituents of cherry laurel (*Prunus laurocerasus*) vinegar, leading to an augmentation in the total flavonoid content in comparison to the untreated vinegar (Yıkmiş et al., 2021). In our investigation, there were observed increments in bioactive components. Ultrasound induces the compression and rarefaction of the medium at a specific point, reaching a critical molecular distance. The surpassing of this distance disrupts the medium, giving rise to cavitation bubbles. The heightened pressure and temperatures resulting from these cavitation bubbles close to the cell surface generate microjets that are directed at the cells, causing the breakdown of cellular structures (Chemat et al., 2011; Perera & Alzahrani, 2021).

Following the RSM, the R^2 value for DPPH exhibited a robust correlation at 99.09% (Table 2). The second-degree polynomial equation for the enhancement of white grape juice is provided in Formula (3).

$$\text{DPPH}(\% \text{inhibition}) = 60.122 - 0.412X_1 - 0.1655X_2 - 0.05351X_1X_1 + 0.000389X_2X_2 + 0.02096X_1X_2 \quad (3)$$

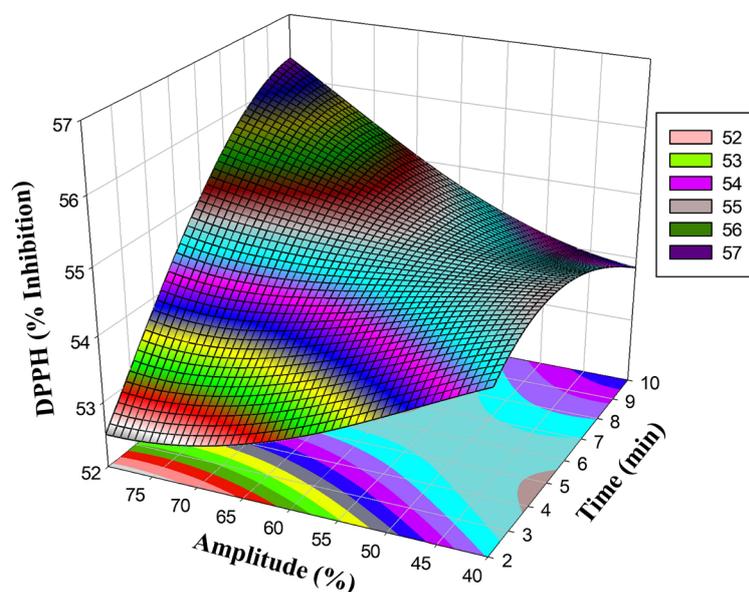


Figure 3. Response surface plots (3D) of DPPH as functions of significant interaction factors

Observations indicate that with the increment in time and amplitude values, there was a corresponding increase in the DPPH (%inhibition). These increments are visually evident in the three-dimensional model graph (Figure 3). Statistically significant disparities were identified in the two-way interaction of the DPPH antioxidant value ($p < 0.05$). Broadly, diverse ultrasound treatments showcased an augmentation in the DPPH value of white grape juice. The DPPH value for the U-WGJ sample is 55.50%, the DPPH value for the C-WGJ sample is $52.18 \pm 0.65\%$ and the DPPH value for the P-WGJ sample is $49.65 \pm 0.57\%$. While ultrasound processing led to an increase in the DPPH value, thermal pasteurization resulted in noteworthy decreases in the DPPH value. Our study aligns with analogous findings reported in research on Amazon fruits (buriti and açai) (de Souza Carvalho et al., 2020), apple beverage enriched with blueberry extracts and black carrot (Brezan et al., 2020) peach and apricot juices after ultrasound treatment (Sattar et al., 2020). Sattar et al., (2020) found that microwave (1.5 min at 850 W) and ultrasound (90 min at 20 kHz) treatment caused a decrease in TPC while increasing DPPH.

CONCLUSION

Grapes cultivated in different regions worldwide are renowned for their health-promoting properties and being an integral part of nutrition due to their abundant antioxidants. This research aimed to optimize the bioactive components in white grape juice using RSM and ultrasound treatment. Additionally, the bioactive values of C-WGJ, U-WGJ and P-WGJ samples were compared. Compared to the C-WGJ sample, U-WGJ samples showed increases of 3.42%, 5.27% and 6.36% in TPC, TFC and DPPH values respectively. The increase in concentrations of bioactive compounds in U-WGJ samples could be attributed to the cavitation pressure induced by ultrasound treatment, resulting in the release of bound bioactive compounds due to the breakdown of fruit cell walls and addition of hydroxyl groups to the phenolic aromatic ring. However, a decrease in bioactive values was observed in P-WGJ samples, possibly due to the high temperature applied during thermal pasteurization damaging the structure of components. RSM results indicated a strong correlation between predicted and experimental values. In summary, optimization of process conditions for ultrasonically treated white grape juice resulted in the enrichment of bioactive components, demonstrating the significant impact of this technology on enhancing bioactive components. This technology stands as an alternative to thermal techniques in food processing due to its advantages in increasing bioactive components.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

M.T.: Conception, Design, Materials, Data Collection and Processing, Analysis and Interpretation, Literature Review, Writing, and Critical Review; N.T.D.: Conception, Materials, Supervision, Analysis and Interpretation, Writing, and Critical Review; S.Y.: Conception, Materials, Supervision, Fundings, Writing, and Critical Review; B.İ.A.: Conception, Literature Review, Writing, and Critical Review.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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