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Optimization of Ultrasound Treatment for Watermelon Vinegar Using Response Surface Methodology: Antidiabetic—Antihypertensive Effects, Bioactive Compounds, and Minerals

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Abstract: Watermelon vinegar is a traditional fermented product with antioxidant activity. This study aimed to investigate the antihypertensive and antidiabetic properties of watermelon vinegar treated through ultrasound using the RSM method. We also evaluated the antioxidant activity (CUPRAC and DPPH), bioactive content (total phenolics and total flavonoids), mineral composition, phenolic compounds, α-glucosidase inhibition %, ACE inhibition %, of optimized, and α-amylase inhibition % during 24 months of storage of optimized watermelon vinegar. Optimized antidiabetic and antihypertensive activity was achieved at 6.7 min and 69% amplitude. The optimization of gallic acid was the dominant phenolic in the optimized ultrasound-treated watermelon vinegar (UT-WV) and showed a significant decrease during the 24 months of storage. The lycopene content of the UT-WV concentrate was 8.36 mg/100 mL, 8.30 mg/100 mL, 7.66 mg/100 mL, and 7.35 mg/100 mL after 0, 6, 2, and 24 months of storage, respectively. The levels of ACE inhibitory activity, α-glucosidase inhibitory activity, and α-amylase inhibitory activity decreased significantly ($p < 0.05$) after 24 months of storage. K, with values of 201.03 ± 28.31, was the main mineral in the UT-WV. Therefore, the bioactive components and the antidiabetic and antihypertensive properties of the UT-WV produced by conventional fermentation were necessary. Therefore, further experimental studies are necessary for a better understanding of the possible and potential health effects of watermelon vinegar.

Keywords: fermented foods; watermelon vinegar; antioxidant activities; non-thermal technologies

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1. Introduction

One of the world's most important commercial crops, watermelon (*Citrullus lanatus*), belongs to the Cucurbitaceae botanical family [1]. Watermelon includes β-carotene, lycopene, polyphenols, mineral salts (especially K, Mg, Ca, and Fe), some vitamins (in particular vitamin C and vitamin A), and dietary fiber [2]. Due to its many bioactive ingredients, watermelon is very popular in the native system of traditional medicine [3]. Watermelon has properties that include analgesic, anti-inflammatory, diuretic, anti-urolithiasis, antioxidant, anti-ulcerative, hypotensive, cardioprotective, laxative activities, prevention of gastrointestinal disorders, hepatic congestion, urinary complaints, and intestinal catarrh [4].

Vinegar is a sour-taste liquid containing acetic acid (about 5% providing characteristic taste and aroma), varying amounts of other fruit acids, coloring agents, salts, and other miscellaneous fermented end products [5]. Vinegar can produce almost all materials containing fermentable sugars that produce alcoholic fermentation by yeasts, followed by ethanol oxidation into acetic acid by acetic acid bacteria [6]. During fermentation, a complex microbial community of microorganisms releases a range of bioactive compounds which, through the modulation of various biochemical pathways, have health benefits [7]. Vinegar is a natural product widely used in traditional medicine and food thanks to its richness in bioactive molecules and physicochemical properties, which is becoming increasingly

important worldwide [8]. Bioactive components such as catechins, gallic acid, chlorogenic acid, caffeic acid, p-coumaric acid, p-hydroxybenzoic acid, and syringic acid have been provided in different vinegars [9,10]. Many researchers have studied the fermentation technology of watermelon vinegar [11–14]. Previously, several research studies showed the beneficial effects of vinegar on blood glucose control, blood pressure and cholesterol reduction, lipid metabolism regulation, antioxidant and antimicrobial effects, and anti-infection properties [15].

Ultrasounds are inaudible high-frequency mechanical waves (20 kHz to 10 MHz) [16]. Due to its environmentally friendly, non-toxic, and safe properties, ultrasonic technology is widely used in the food industry [17]. Ultrasound technology is generally used in two main areas in the food industry: food processing and food properties measurement [18,19]. Ultrasound technology is recognized as one of several promising food processing technologies [20]. Many researchers have found that treatment with ultrasound is a good and promising technology, which causes a minimal reduction in the bioactive components of the food [21–25]. Studies using ultrasound technology on vinegar show minimal effects on losing quality properties [26]. In food production areas, in addition to sterilization, freezing, extraction, and drying, ultrasound, a green technology, can also be used [27].

Diabetes mellitus is an important and significant human disease composed of multiple clinical manifestations [28]. In animals, watermelon juice has antidiabetic activity potential (experimental diabetic model) [29]. Researchers have indicated that vinegar inhibits amylases and reduces postprandial blood glucose levels [30,31]. Hypertension is a risk factor for heart failure associated with decreased life expectancy, stroke, and heart attack [32]. Angiotensin-converting enzyme (ACE) is an effective bioactive in regulating blood pressure, and much research into it has been carried out in recent years [33–35]. Watermelon consumption can increase nitric oxide bioavailability, preventing atherosclerosis, arterial stiffening, and the development of hypertension [36]. Saqib et al. (2022) reported that watermelon seeds contain ISO-induced myocardial infarction and have significant antihypertensive properties [37].

In the literature, there are several research articles dealing with ultrasound treatment. However, a review of the literature shows that there have been no studies on the use of ultrasound technology to optimize the antidiabetic and antihypertensive effects of watermelon vinegar. This study aimed to investigate the anti-diabetes and antihypertensive properties of ultrasound-treated watermelon vinegar using the RSM method. Additionally, we evaluated the bioactive content (total phenolics and total flavonoids), mineral composition, phenolic compounds, antioxidant activity, α-amylase inhibitory activity %, ACE inhibitory activity %, and α-glucosidase inhibitory activity % during 24 months of storage of optimized watermelon vinegar.

2. Materials and Methods

2.1. Vinegar Preparation

Watermelons (*Citrullus lanatus*) from Tekirdağ, Turkey, were used to produce vinegar. They were washed with water. The rind was cut off and the red part taken apart. Then, the seeds were removed. The traditional vinegar method was explained before by Yıkmış, 2019. *Saccharomyces cerevisiae* yeast was inoculated into the mixture at a rate of 0.4% for the initial fermentation of the production in compliance with environmental hygiene and microbiological regulations. The fermentation was allowed to take place at 28 °C and was terminated after 24 days. The second fermentation was inoculated with an acetic acid culture (5%). This was performed at 28 °C for 60 days. The analysis of the second fermentation showed an acetic acid content of approximately 4%, and vinegar fermentation was stopped by removing the cellulosic microorganism known as the vinegar mother. The sample of watermelon vinegar was stored at -20 ± 1 °C.

2.2. Ultrasound Treatment

An ultrasonic was used for ultrasound treatment (26 kHz, Hielscher Ultrasonics Model UP200St, Berlin, Germany). Ultrasound parameters are 40%, 55%, 70%, 85%, and 100% amplitudes, and 2, 5, 8, and 11 min processing durations are in constant mode. The ice-water bath prevented overheating. At the end of the ultrasound treatment, the watermelon vinegar samples were immediately cooled and stored at -18 ± 1 °C until analysis (UT-WV).

2.3. Experimental Design

The Response Surface Method (RSM) was used to investigate the effect of ultrasound technology on the antihypertensive and antidiabetic properties of watermelon vinegar. Then, the Minitab Statistical Analysis Software (Minitab 18.1.1 version, State College, PA, USA) was used to analyze the data. A five-level experimental design with two factors was set up. Durations in the range of X_1 (time) and X_2 (amplitude) were determined as independent variables. The equation models were constructed using the following quadratic polynomial formula:

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

This formula can be defined in the following way: the intercept term (β_0; the first order (linear) equation coefficient (β_i); the quadratic equation coefficient (β_{ii}); the two-factor cross-interaction coefficient (β_{ij}); the dependent variable (y); and independent variables X_i and X_j.

2.4. Angiotensin-Converting Enzyme Inhibition Assay

The ACE inhibitory activity was determined according to a modification of the method of Cushman and Cheung, 1971 [38], as follows. The reaction mixture contained 100 µL of ACE solution (2.5 mU/mL), 50 µL of the sample solution, and 50 µL of 8 mM HHL as a substrate. Processed at 37 °C for 90 min. After the addition of 250 µL of 1 N HCl, the reaction was terminated. The hippuric acid was redissolved in an aqueduct. A UV-VIS spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Melbourne, Australia) was used to measure the absorbance of the samples (228 nm). The ACE inhibitory activity was calculated as follows:

$$Inhibition\ activity\ (\%) = \left[\frac{(A_c - A_s)}{(A_c - A_b)}\right] \times 100 \quad (2)$$

where before A_b is the absorbance when the stop solution has been added prior to before the start of the reaction (blank), A_s is the absorbance of the reaction mixture (sample), and A_c is the absorbance of the buffer (control). The concentration of the extract was defined to reduce 50% of ACE activity. To lower 50% of ACE activity, the concentration of the extract was defined as the IC50.

2.5. Inhibition of α-Amylase Enzyme

α-Amylase activity was assayed using an established method with minor changes [39]. Acarbose was used as the positive control. Briefly, a sample of acarbose (0.2 mL) was mixed with α-amylase (40 mL, 5 U/mL) and sodium phosphate buffer (0.36 mL, 0.02 M, pH 6.9). After an incubation period of 20 min at 37 °C, starch solution (300 mL, 1%) was put in a sodium phosphate buffer (0.02 M), followed by adding 0.2 mL DNS. The mixture content was kept in a boiling water bath (5 min). Add 6 mL distilled water. A UV-VIS spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Melbourne, Australia) was used to measure the absorbance (540 nm). The inhibition activity was calculated using the following equation:

$$\%\ Inhibition = \left(1 - \frac{A_s}{A_c}\right) \times 100 \quad (3)$$

With A_s and A_c being the absorbance for the control and sample, respectively. Logarithmic regression analysis was used to calculate the IC_{50} values of the pure compounds.

2.6. Inhibition of α-Glucosidase Enzyme

A-glucosidase inhibition was performed according to the modified method of Zhang et al. (2011) [39]. Briefly, the sample (50 mL) was mixed with enzyme (50 µL, 0.57 U/mL) and incubated at 37 °C for 10 min. Subsequently, α-pNPG (50 µL, 5 mM) was added. After, the mixture was then incubated for 20 min at 37 °C., Na_2CO_3 (1 M, 50 µL) was added. A UV-VIS spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Melbourne, Australia) was used to measure the absorbance (405 nm). The inhibition activity was calculated using the following equation:

$$\% \, Inhibition = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100 \quad (4)$$

$A_{control}$ and A_{sample} are the absorbance of the sample and control, respectively. IC_{50} values of pure compounds were determined.

2.7. Storage Study

To determine the total phenolics and flavonoids content, phenolic compounds, minerals, and antioxidant capacity (CUPRAC and DPPH) of the UT-WV samples obtained after RSM optimization, the vinegar samples were kept (glass jars) for 0, 6, 12, and 24 months in room conditions without direct light.

2.8. Determination of Lycopene

The modified method was used to determine the lycopene concentration [40]. Weigh about 0.6 g of the watermelon vinegar sample and add 5 mL of 0.05% (*w*/*v*) BHT in acetone/ethanol (5 mL, 95%) and hexane (10 mL). The mixture was centrifuged (400 g, 15 min). After that, distilled water (3 mL) was added. The vials were left to phase separation at room temperature for 5 min. Using a spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Melbourne, Australia), the absorbance of the upper hexane layer was measured at 503 nm in a 1 cm path-length quartz cell. Hexane was used as a blank. The lycopene concentration (mg/L) was calculated as follows:

$$\text{Lycopene} = Abs_{503} \times MW \times DF \times 1000/\varepsilon \times L \quad (5)$$

where DF is the dilution factor, L is the path length in cm, ε is the molar extinction coefficient for lycopene (172,000 L/mol/cm), and MW is the molecular weight of lycopene (536.9 g/mol).

2.9. Contents of Total Phenolics and Flavonoids

The total phenolic content result was recorded using the Folin-Ciocalteau method [41]. Watermelon vinegar, distilled water, and Folin–Ciocalteu reagent were combined in an aliquot of 50 µL, 450 µL, and 2.5 mL, respectively. After waiting 5 min in darkness, saturated sodium carbonate (2 mL) was added. Then, the absorbance was measured at 765 nm using a spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Melbourne, Australia). The results are shown as mg gallic acid equivalent/100 g.

The total flavonoid result of the watermelon vinegar was carried out using the colorimetric technique, according to the study by Zhishen et al. (1999) [42]. After preprocessing, the absorbance results were read at 510 nm. The results are shown as mg catechinic values (CE)/L.

2.10. Determination of Total Antioxidant Capacity by CUPRAC and DPPH

The antioxidant activity was investigated using two methods: cupric ion reducing antioxidant capacity (CUPRAC) and the scavenger 2,2-diphenyl-1-picrylhydrazyl (DPPH)

radical. Apak et al. (2006) [43] and Grajeda-Iglesias et al. (2016) [44] methodologies were used to determine CUPRAC and DPPH, respectively.

2.11. Determination of Total Monomeric Anthocyanin

Total monomeric anthocyanin (TAC) was determined using the pH differential method. Briefly, samples (1 mL) buffer (pH: 1.0) were added, and samples (1 mL) buffer (pH: 1.0) were added to the first tubes. Samples (1 mL) and buffer (9 mL, 4.5 pH) were added to the second tube. After waiting 15 min, the absorbance values of the samples were measured using a SP-UV/VIS-300SRB spectrophotofmeter (against water at 510 nm and 700 nm). The total monomeric anthocyanin (mg/L) was calculated as follows [45,46]:

$$\text{Total monomeric anthocyanin (mg/L)} = \text{A MW Df } 1000/(\varepsilon)\ \ell \quad (6)$$

$$\text{A} = (\text{A}\lambda_{\text{vis-max}} - \text{A}_{700})\text{pH } 1.0 - (\text{A}\lambda_{\text{vis-max}} - \text{A}_{700})\text{pH } 4.5 \quad (7)$$

ε = cyanidin-3-glucoside absorption coefficient (26,900 L/(cm mol))
Df = Dilution factor
MW = cyanidin-3-glucoside (cyd-3-glc) molecular weight: 449.2 (gmol/L)
ℓ = Lightpath (1 cm).

2.12. Phenolic Compounds

The chromatographic process for detecting phenolic compounds was made according to the study recognized by Portu et al. (2016) [47]. C-18, ACE Generix column (250 × 4.6 mm; 5 µm packing; Agilent, Waldbronn, Germany) (Advanced Chromatography Technologies Ltd., Aberdeen, Scotland) was used in the detection analysis. Phenolic compound concentrations are expressed as µg/mL.

2.13. Mineral Content

A simultaneous inductively coupled plasma—optical emission spectrometer (ICP-OES) device (Thermo Scientific iCap 6000 Dual view, Cambridge, England) was used to evaluate the watermelon vinegar mineral content. Cobalt (Co), calcium (Ca), iron (Fe), Aluminum (Al), manganese (Mn), silver (Ag), magnesium (Mg), copper (Cu), zinc (Zn), chromium (Cr), nickel (Ni), lead (Pb), and sodium (Na) minerals were analyzed in watermelon vinegar. The data are expressed as mg/L samples for each mineral [48].

2.14. Statistical Analysis

The results of the present study are expressed as the mean of three replicates ± standard error. One-way analysis of variance (ANOVA) was performed. All data were examined using the SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) and SigmaPlot 12.0 Statistical Analysis Software (Systat Software, Inc., San Jose, CA, USA). A comparison of the mean values of the samples was conducted using Tukey's test.

3. Results and Discussion

3.1. Modeling Antidiabetic and Antihypertensive Effects

The experimental and predicted results of the ultrasound effects applied to the antidiabetic and antihypertensive watermelon vinegar samples are given in Table 1. A second order polynomial regression model was used to analyze the experimental data from the study. The antidiabetic and antihypertensive quadratic polynomial regression equations from the RSM modelling are shown below (Time; X_1, Amplitude; X_2).

$$\text{ACE Inhibitory Activity\%} = 2.33 + 0.376X_1 + 0.9147X_2 - 0.18173X_1^2 - 0.007241X_2^2 + 0.03044X_1X_2 \quad (8)$$

$$\alpha - \text{Amylase Inhibitory Activity\%} = 5.83 + 1.036X_1 + 0.9247X_2 - 0.008537X_1^2 - 0.009593X_2^2 + 0.03289X_1X_2 \quad (9)$$

$$\alpha - \text{Glucosidase Inhibitory Activity\%} = 1.84 + 1.786X_1 + 1.063X_2 - 0.2395X_1^2 - 0.008803X_2^2 + 0.02095X_1X_2 \quad (10)$$

Table 1. Experimental and predicted responses of RSM results of UT-WV.

Run no.	Independent Variables		Dependent Variables					
	Time (X_1)	Amplitude (X_2)	ACE Inhibitory Activity %		α-Amylase Inhibitory Activity %		α-Glucosidase Inhibitory Activity %	
			Experimental Data	RSM Predicted	Experimental Data	RSM Predicted	Experimental Data	RSM Predicted
1	11 (+1)	55(−1)	21.78 ± 0.42	21.95	36.59 ± 1.27	33.43	32.96 ± 1.14	37.02
2	11 (+1)	85 (+1)	24.15 ± 1.15	23.93	38.85 ± 1.45	36.17	36.36 ± 0.70	38.85
3	8(0)	70 (0)	27.38 ± 0.55	27.41	42.92 ± 1.42	40.24	40.12 ± 0.96	43.81
4	2 (−1.41)	70 (0)	23.34 ± 1.36	23.27	39.21 ± 1.36	34.45	34.45 ± 1.19	38.66
5	8(0)	70 (0)	27.35 ± 0.18	27.41	44.27 ± 1.42	40.24	40.32 ± 1.40	43.81
6	8(0)	70 (0)	27.35 ± 0.58	27.41	44.27 ± 0.94	40.24	40.32 ± 1.08	43.81
7	8(0)	70 (0)	27.35 ± 0.58	27.41	44.27 ± 0.71	40.24	40.32 ± 1.55	43.81
8	14 (+1.41)	70 (0)	18.43 ± 1.55	18.46	31.64 ± 1.10	28.94	29.03 ± 1.01	31.71
9	5 (−1)	85 (+1)	23.71 ± 0.67	23.60	39.83 ± 1.21	35.97	36.27 ± 1.26	40.44
10	8 (0)	100 (+1.41)	19.95 ± 0.94	20.13	35.85 ± 0.87	32.34	32.05 ± 1.95	35.83
11	5 (−1)	55 (−1)	26.82 ± 0.46	27.10	41.34 ± 1.43	39.15	38.79 ± 0.77	42.38
12	8 (0)	70 (0)	27.69 ± 1.25	27.41	44.27 ± 1.42	40.24	40.32 ± 1.40	43.81
13	8 (0)	40 (−1.41)	21.87 ± 0.25	21.65	36.41 ± 0.86	32.78	33.16 ± 1.15	35.94
UT-WV	6.7	69	27.72		40.50		44.17	
Experimental values			26.70 ± 0.67		38.56 ± 1.34		42.35 ± 0.48	
% Difference			3.68		4.86		4.19	

ACE: Angiotensin I converting enzyme; UT-WV: ultrasound-treated watermelon traditional vinegar; RSM: Response surface methodology. Results are presented as mean ± standard deviation (n = 3).

Table 2 shows the analysis of variance (ANOVA) for α-amylase inhibitory activity %, ACE inhibitory activity %, and α-glucosidase inhibitory activity %. According to Table 2, for antidiabetic and antihypertensive effects, the quadratic (2nd degree) and two-way interaction functions were found to be statistically significant ($p < 0.05$). During the determination of the optimization prediction height, model incompatibility tests were performed for the values of α-amylase inhibitory activity %, ACE inhibitory activity %, and α-glucosidase inhibitory activity % of the ultrasound-independent factors. The adjusted R^2, the R^2, the standard deviation, and the predicted R^2 values of the functions have been examined. According to these results, the α-amylase inhibitory activity, ACE inhibitory activity, and α-glucosidase inhibitory values were determined as 99.72%, 99.58%, and 98.08%, respectively. According to these results, the RSM model has a high ability to predict antidiabetic and antihypertensive effects. At the same time, 3D mash graphs of the watermelon vinegar samples and linear comparisons of the experimental and RSM predicted values are given in Figure 1. When the linear graphs were examined, it was seen that there was a high R^2 correlation.

Table 2. Corresponding *p*-values of linear, interaction, and quadratic terms of regression coefficients obtained by RSM of responses for α-amylase inhibitory activity, ACE inhibitory activity, and α-glucosidase inhibitory activity.

Source	DF	ACE Inhibitory Activity %		α-Amylase Inhibitory Activity %		α-Glucosidase Inhibitory Activity %	
		F-Value	*p*-Value	F-Value	*p*-Value	F-Value	*p*-Value
Model	5	496.75	0.000	334.6	0.000	71.34	0.000
Linear	2	194.67	0.000	107.46	0.000	33.53	0.000
X_1	1	353.66	0.000	213.55	0.000	67.05	0.000
X_2	1	35.68	0.001	1.37	0.279	0.02	0.892

Table 2. *Cont.*

Source	DF	ACE Inhibitory Activity %		α-Amylase Inhibitory Activity %		α-Glucosidase Inhibitory Activity %	
		F-Value	*p*-Value	F-Value	*p*-Value	F-Value	*p*-Value
Square	2	970.6	0.000	688.19	0.000	141.54	0.000
X_1^2	1	1250.97	0.000	975.08	0.000	196.82	0.000
X_2^2	1	1241.43	0.000	788.19	0.000	166.12	0.000
2-Way Interaction	1	153.23	0.000	81.68	0.000	6.57	0.037
$X_1 * X_2$	1	153.23	0.000	81.68	0.000	6.57	0.037
Error	7						
Lack-of-Fit	3	3.8	0.115	31.02	0.003	2.16	0.236
Pure Error	4						
Total	12						
R^2		99.72%		99.58%		98.08%	
Adj R^2		99.52%		99.29%		96.70%	
Pred. R^2		97.79%		95.99%		89.36%	

X_1: Time; X_2: Amplitude; DF: Degree of freedom; ACE: Angiotensin I converting enzyme; R^2: correlation coefficient; Adj R^2: Adjusted-R; Pred. R^2: Predicted-R^2. *p*-values less than 0.05 indicate that model terms are significant.

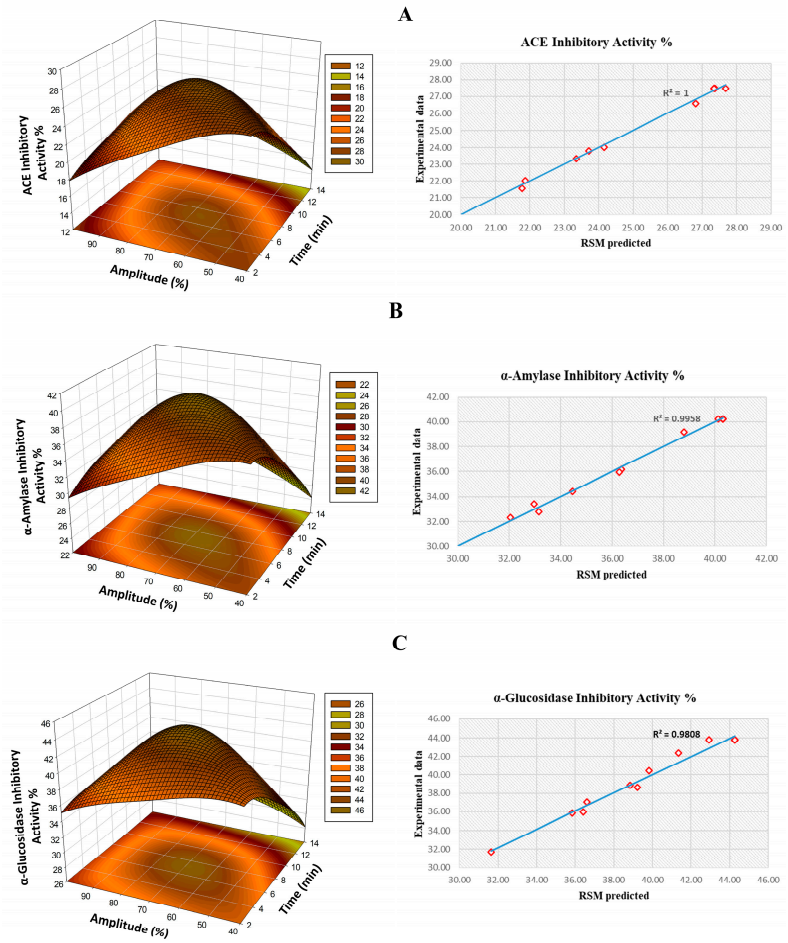

Figure 1. Response surface plots (3D) and linear for ACE inhibitory activity (**A**), α-amylase inhibitory activity (**B**), and α-glucosidase inhibitory activity (**C**) analysis as a function of significant interaction factors for RSM.

As a result of optimizing the ultrasound process, its parameters were determined to have a 6.7 min and 69% amplitude for the variables X_1 and X_2, respectively. As a result of the best ultrasound, the antidiabetic and antihypertensive effects of watermelon vinegar are strengthened. The α-amylase inhibitory activity, ACE inhibitory activity, and α-glucosidase inhibitory values were determined as 27.72%, 40.53%, and 44.20%, respectively. The effects of the model as a result of the experiment and optimization were measured and compared again. The percentage differences in the α-amylase inhibitory activity, ACE inhibitory activity, and α-glucosidase inhibitory values were determined as 3.68%, 4.86%, and 4.19%, respectively. The low differences between the RSM optimization and experimental results showed the success of the model prediction. In explaining the mechanism of action of vinegar on blood sugar development, increased glucose uptake, the inhibition of transcription factors, and the alpha-amylase effect may be effective [49]. The ACE inhibitor effect of vinegar on mice was examined, and it was reported that acetic acid caused hypertensive effects [50]. The antihypertensive and antidiabetic results of the ultrasound-enhanced watermelon vinegar were found to show similar effects in the study of tomato vinegar [51], organic *Cornus mas* L. (cornelian cherry) vinegar [52], and fresh pomegranate juice [53]. While ultrasound treatments increase the antihypertensive and antidiabetic effects, the release of bioactive compounds due to reactions occurring with acoustic cavitation may explain the increases.

3.2. Antioxidant Activities

Pearson correlation results of the data of the samples during storage conditions are given in Figure 2. The DPPH and CUPRAC results of the UT-WV samples under the storage conditions are shown in Figure 3. Two antioxidant indexes were selected to study the optimized watermelon vinegar's antioxidant capacities, including CUPRAC and DPPH radical scavenging activity. Further storage of the UT-WV for 24 months caused significant reductions ($p < 0.05$) in CUPRAC and DPPH, as shown in Figure 3. In contrast with our results, an increase in antioxidant activity was detected in wood vinegar stored in darkness for two years. The researchers indicated that phenolic compounds were the primary active substances that were significant scavenging free radicals [54]. Muzaffar et al. (2016) found that the antioxidant activity increased in ultrasound-treated cherry samples with a storage time of 15 days at 4 °C [55]. Oms-Oliu et al. (2009) reported that the lycopene content was highly correlated with the antioxidant capacity retention ($R^2 = 0.964$) of high-intensity pulsed electric field-treated watermelon juice [40]. The significant correlations were DPPH–Oleuropein (0.96), DPPH–lycopene (0.96), DPPH–TPC (0.97), and DPPH–TAC (0.95). Finally, positive correlations among CUPRAC and bioactive compounds were found in some cases; e.g., DPPH (r = 0.98), TFC (r = 0.98), and lycopene (r = 0.99) (Figure 2). These results were in line with the results stated by Wang et al. (2022), who indicated that the polyphenols and flavonoids of apple peels and persimmon vinegar were suggested to be positively correlated with the DPPH scavenging activity [56].

3.3. Bioactive Compounds

Lycopene is a carotenoid with significant antioxidant properties, providing the red color of watermelon [57]. Lycopene from watermelon is a radical-scavenging pigment that protects against certain cancers [58]. Quek et al. (2007) reported that lycopene was 48.13 ± 1.21 (μg/mL), 36.45 ± 2.05 (μg/g), and 954.02 ± 3.11 (μg/g) of watermelon juice, watermelon, and spray-dried watermelon powders, respectively [59]. The lycopene content in the ultrasound-treated watermelon vinegar concentrate was 8.36 mg/100 mL, 8.30 mg/100 mL, 7.66 mg/100 mL, and 7.35 mg/100 mL during storage for 0, 6, 2, and 24 months, respectively. The values showed a significant reduction in the 24-month storage lycopene results compared to the beginning of storage. This agrees with the findings of Acharya et al. (2021), who reported that the lycopene content decreased after 20 days of storage in watermelon [60]. Similarly, Fish and Davis (2003) reported that lycopene decreased by 30–40% in watermelon (Sangria cultivar) in 12-month storage at −20 °C [61].

A similar loss in lycopene was reported for fresh-cut watermelon stored after 7 days at 2 °C [62]. The lycopene degradation rate could vary depending on factors such as oxygen, pH, moisture content, temperature, solvent polarity, and soluble solids during storage [63]. In another study, the lycopene content peaked for 7 days in grafted watermelon (Celebration, Gallery, Pegasus, and Torpilla) [64]. Seasons, cultivars, and production sources can affect the lycopene content [65].

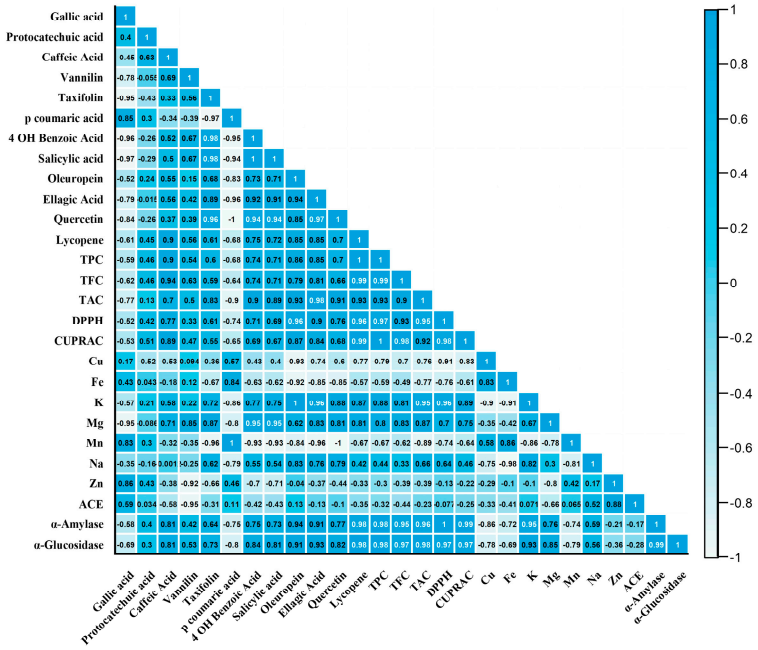

Figure 2. Pearson correlation relationship between samples' antioxidant activity, phenolic compounds, bioactive compounds, mineral, antidiabetic and antihypertensive values.

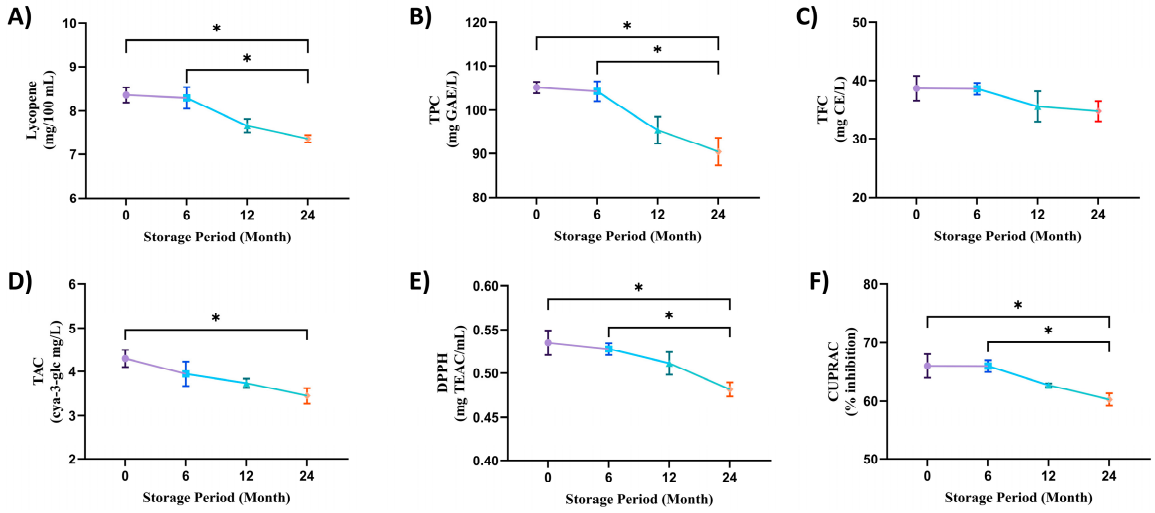

Figure 3. Results for lycopene (**A**), total phenolic compounds (**B**), total flavonoid compounds (**C**), Total anthocyanin content (**D**), DPPH (**E**), and CUPRAC (**F**) during storage of the ultrasound-treated watermelon traditional vinegar sample. Values with different letters in the rows are significantly different (* $p < 0.05$). Results are presented as mean ± standard deviation (n = 3).

Anthocyanins are flavonoids commonly found in fruits that exert strong antioxidant activity. Bioactive compounds such as anthocyanin and TPC enhance food quality, such as color and taste, and play an essential role in human wellness [66]. Anthocyanins are provided in purple, blue, or red colors for fruit [67]. The results revealed that the TAC content

was significantly ($p < 0.05$) decreased to 3.45 mg/100 mL compared to 4.29 mg/100 mL after 24 months of storage. Similar to the present study, Choi et al. (2002) reported that total anthocyanin in reconstituted blood orange (*Citrus sinensis*) juice decreased (25%) after 7 weeks at 4.5 °C [68].

Polyphenols have a high antioxidant capacity, anti-inflammatory, anticancer, antibacterial, and antiviral properties [69]. The TPC, TFC, and TAC results of the UT-WV samples under the storage conditions are shown in Figure 3. Feng et al. (2023) reported that the total phenols were 203.05 ± 2.04 mg/mL and total flavonoids 7.49 ± 1.28 mg/mL in watermelon vinegar [14]. After 24 months of storage, the TPC values decreased significantly in the UT-WV. The TFC content did not change, especially when stored for 24 months. However, analyses of the UT-WV samples showed a loss of ∼10% TFC over 1 year of storage. A study by Duan et al. (2019) on Zhenjiang aromatic vinegar found that the TPC and TFC increased by the 24th month compared with the 0th day, contrary to our study [70]. Rawson et al. (2011) found that watermelon juice had phenolic and lycopene contents of 13.89 mg GAE/100 mL and 5.29 mg/100 mL. They reported degradation in the total phenolic and lycopene content as the ultrasound treatment time increased (upper 10 min) [71]. Makroo et al. (2017) reported that the ohmic heating process also causes a decrease in total phenolic compounds of watermelon juice [58]. The Pearson's positive coefficient of determination (R^2) predicted among the TPC and TFC were significantly correlated with caffeic acid (0.9, 0.94) and lycopene (1, 0.99). TAC was significantly positively correlated with 4_OH Benzoic Acid (0.90), Oleuropein (0.93), and Ellagic Acid (0.98) (Figure 2).

3.4. Phenolic Compounds

Watermelon is a good source of phenolics, mainly hydroxycinnamic acid derivatives [71]. Phenolic compounds are due to specific chemical changes during acetic acid fermentation. The level of phenolic acids' component changes during different stages of fermentation in vinegar production [72]. The samples were observed at 0, 6, 12, 18, and 24 months to determine the phenolic compounds of the UT-WV samples obtained after optimization. It was found that the watermelon vinegar contained 11 kinds of polyphenols: gallic acid, protocatechuic acid, caffeic acid, vanniline, taxifolin, ellagic acid, p_coumaric acid, 4-hydroxybenzoic acid, salicylic acid, oleuropein, quercetin, and flavone. Gallic acid was the dominant phenolic in watermelon vinegar, and significant differences were detected in the 24-month storage samples compared to the beginning sample; the values were 34.89 ± 0.46 µg/mL and 29.76 ± 0.49 µg/mL, respectively. Gallic acid is a phenolic antioxidant with multiple pharmacological activities, including antioxidant, antibacterial, antifungal, anti-inflammatory, photoprotective, anticarcinogenic, and antityrosinase [73]. Contrary to our study, in one study, catechin 159 × 10^{-7} mg/mL was the most abundant phenolic component in watermelon vinegar [74]. As shown in Table 3, the relative content of polyphenols in 0-, 6-, 12-, and 24-month storage vinegar differed, but they all contained high gallic acid contents. These results are similar to the results obtained in ultrasound-treated horsetail-fortified traditional apple vinegar by Tokatlı Demirok et al. (2023), who reported that protocatechuic acid, caffeic acid, vanniline, taxifolin, 4-hydroxybenzoic acid, and quercetin are reduced after 24 months of storage [75]. Following a further 6-month storage, no significant differences were detected in the UT-WV, but the storage of samples for 12 and 24 months caused significant ($p < 0.05$) losses of caffeic acid (Table 3). Duan et al. (2019) found that vanillin, *p*-hydroxybenzoic acid, and catechin were significant compounds in Zhenjiang aromatic vinegar. They indicated that these contents reached the highest level (148.378 ± 11.98, 27.061 ± 2.690, and 57.453 ± 3.740 mg/L, respectively) in the sixth year of the aging process, and then decreased [70]. Similarly to our study, Liu et al. (2019) found that gallic acid, caffeic acid, and protocatechuic acid were the most abundant, at 12.56 ± 0.86 µg/mL, 3.29 ± 0.05 µg/mL, and 3.58 ± 0.14 µg/mL, respectively [76]. Chen et al. (2020) reported that the p-coumaric acid content significantly increased, and ranged between 0.018 ± 0.0033 mg L^{-1} and 0.027 ± 0.0001 mg L^{-1} during the 9-day acetic acid fermentation process in sugarcane vinegar, which is in line with our study [72]. Posi-

tive correlations were found between 4-hydroxybenzoic acid and salicylic acid with Mg (0.95), while negative correlations were found between gallic acid and Mg (−0.95).

Table 3. Changes in phenolic compounds during storage of ultrasound-treated watermelon traditional vinegar.

Phenolic Compound	Storage Period (Month)			
	0	**6**	**12**	**24**
Protecatechuic Aldehyde	<0.027	<0.027	<0.027	<0.027
Gallic acid	29.76 ± 0.49 ^{a}	34.41 ± 0.36 ^{b}	35.84 ± 0.88 ^{b}	34.89 ± 0.46 ^{b}
Protecatechuic Acid	10.27 ± 0.27 ^{a}	15.93 ± 0.08 ^{c}	12.36 ± 0.04 ^{b}	10.19 ± 0.05 ^{a}
Catechin	<0.01	<0.01	<0.01	<0.01
Sesamol	<0.034	<0.034	<0.034	<0.034
Syringic Acid	<0.104	<0.104	<0.104	<0.104
Epicatechin	n.d	n.d	n.d	n.d
Caffeic Acid	0.23 ± 0.00 ^{b}	0.24 ± 0.01 ^{b}	0.18 ± 0.01 ^{a}	0.18 ± 0.00 ^{a}
Ferulic Acid	<0.064	<0.064	<0.064	<0.064
Vanniline	0.84 ± 0.03 ^{a}	0.69 ± 0.01 ^{b}	0.01 ± 0.01 ^{a}	0.58 ± 0.01 ^{c}
Taxifolin	1.59 ± 0.06 ^{a}	1.06 ± 0.05 ^{b}	1.13 ± 0.04 ^{b}	1.07 ± 0.02 ^{b}
p_coumaric Acid	0.06 ± 0.08 ^{a}	0.59 ± 0.01 ^{ab}	0.52 ± 0.01 ^{b}	0.73 ± 0.02 ^{c}
Rosmarinic Acid	<0.003	<0.003	<0.003	<0.003
4-hydroxybenzoic acid	20.17 ± 0.06 ^{a}	13.41 ± 0.26 ^{b}	11.63 ± 0.25 ^{c}	11.73 ± 0.12 ^{c}
Salicylic acid	13.86 ± 0.13 ^{a}	9.48 ± 0.06 ^{b}	8.32 ± 0.04 ^{c}	8.45 ± 0.06 ^{c}
Oleuropein	0.23 ± 0.01 ^{a}	0.15 ± 0.00 ^{b}	0.15 ± 0.01 ^{b}	0.01 ± 0.01 ^{c}
Rezveratrol	<0.019	<0.019	<0.019	<0.019
Routine	<0.022	<0.022	<0.022	<0.022
Quercetin	2.79 ± 0.03 ^{a}	1.93 ± 0.02 ^{b}	2.03 ± 0.03 ^{b}	1.65 ± 0.06 ^{c}
Kaempferol	n.d.	n.d.	n.d.	n.d.
Ellagic Acid	0.67 ± 0.04 ^{a}	0.44 ± 0.01 ^{b}	0.42 ± 0.01 ^{b}	0.23 ± 0.02 ^{c}
Flavone	<0.057	<0.057	<0.057	<0.057

Values with the different letters within the line are significantly different ($p < 0.05$). Results are presented as mean ± standard deviation (n = 3). n.d. = not detected.

3.5. Antidiabetic and Antihypertensive Effects

The antidiabetic and antihypertensive effects are shown in Figure 4. Storage for 24 months significantly ($p < 0.05$) decreased the level of α-Amylase inhibitory activity by about 13.09% when compared to 0-month storage. Wen et al. (2023) indicated that pickled tea (30 mg/mL) inhibited 50.1 ± 2.0% of α-amylase enzymatic activity, and the inhibitory rate increased after 2 months of fermentation but decreased after 8 months [77]. Yim et al. (2015) found that the α-glucosidase inhibitory ability of *Cudrania tricuspidata* (Kujippong) vinegar was shown to be 91.4% after 72 h of fermentation [78]. Positive correlations among α-Amylase inhibitory activity and bioactive compounds were found in some cases; e.g., DPPH (1), TPC (0.98), lycopene (0.98), TAC (0.96), and TFC (0.95).

In the UT-WV, the α-Glucosidase inhibitory activity decreased over time, whose inhibitory activity decreased ($p > 0.05$) following up to 6 months of storage, and, after this time, there was a significant reduction ($p < 0.05$) in inhibitory activity. Rasouli et al. (2017) speculated that caffeic acid, quercetin, curcumin, epicatechin, cyanidin, resveratrol, daidzein, ferulic acid, hesperetin, eridyctiol, syringic acid, narenginin, and pinoresinol

were the most potent α-glucosidase inhibitory activity % [79]. Similar to this study, it was determined that caffeic acid and quercetin decreased with storage, while α-glucosidase inhibitory activity % decreased in parallel.

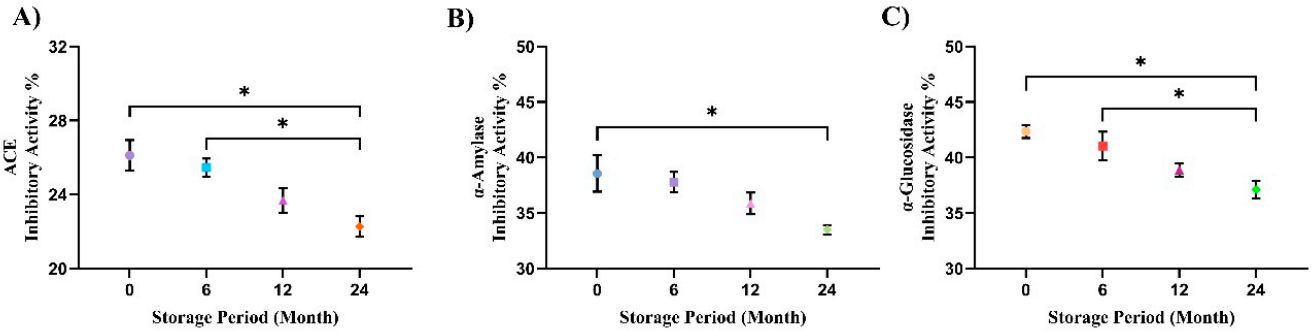

Figure 4. ACE inhibitory activity % (**A**), α-amylase inhibitory activity % (**B**), and α-glucosidase inhibitory activity % (**C**) of the ultrasound-treated watermelon traditional vinegar sample. Letters atop bars indicate statistically significant differences (ns: no significant; * $p < 0.05$; (n = 3 ± SD).

The ACE Inhibitory activity, α-amylase inhibitory activity, and α-glucosidase inhibitory activity content of the UT-WV are presented in Figure 4. The level of ACE inhibitory activity % in the ultrasound-treated watermelon vinegar was 26.12 and gradually decreased with the storage time; e.g., there was a 14.73% decrease at the end of the 24 months. Negative correlations were found among ACE with vannilin (−0.95). Kim et al. (2023) reported that most of the acetic acid bacteria isolated from eight vinegar samples had higher ACE inhibitory activity than the 0.1% captopril-positive control (76.9%) [34]. Similarly to the present study, Morgan et al. (2016) observed the protective effect of apple cider vinegar on type II diabetes management [35]. The antidiabetic mechanism of ultrasound treatment in watermelon vinegar can be explained by the parallel in the amount of bioactive components due to cavitation, as reported by Yıkmış et al. (2022) [53].

3.6. Minerals

The results of the mineral contents analyses of the UT-WV sample are shown in Figure 5. The main mineral present in UT-WV was K, with values of 201.03 ± 28.31. The K values changed after 6, 12, and 24 months of storage: 188.25 ± 13.52, 186.93 ± 4.89, and 165.14 ± 3.74, respectively. The mean concentrations of macro minerals (K, Mg, and Na) and watermelon's microminerals (Mn, Fe, and Zn) were 201.03 ± 28.31 mg/L, 9.91 ± 2.22 mg/L, 4.58 ± 0.64 mg/L and 0.35 ± 0.15 mg/L, 0.69 ± 0.1 mg/L, and 0.12 ± 0.03 mg/L, respectively [80]. No significant variation in Cu, Fe, K, Mg, and Zn content was seen during the 24 months of storage. Further storage of UT-WV for 24 months caused significant reductions ($p < 0.05$) in Mn, as shown in Figure 5. The results also demonstrated that the Cu values were fairly consistent for up to twelve months of storage. Similar to the present study, Ozturk et al. (2015) found that K, Ca, and Na were the most abundant minerals present in the twenty traditional home-made vinegars [81]. In addition, Antoniewicz et al. (2022) studied homemade grape vinegar. They found that K, P, Ca, Mg, and Na were the main minerals in the vinegar samples [82]. Moreover, Chou et al. (2015) reported that Mg and K were the main mineral compounds, while Fe, Se, Ca, and Mn were also analyzed in black vinegar [83]. Negative correlations among oleuropein with Cu (−0.93) and Fe (−0.92) and positive correlations among oleuropein with K (1) were found.

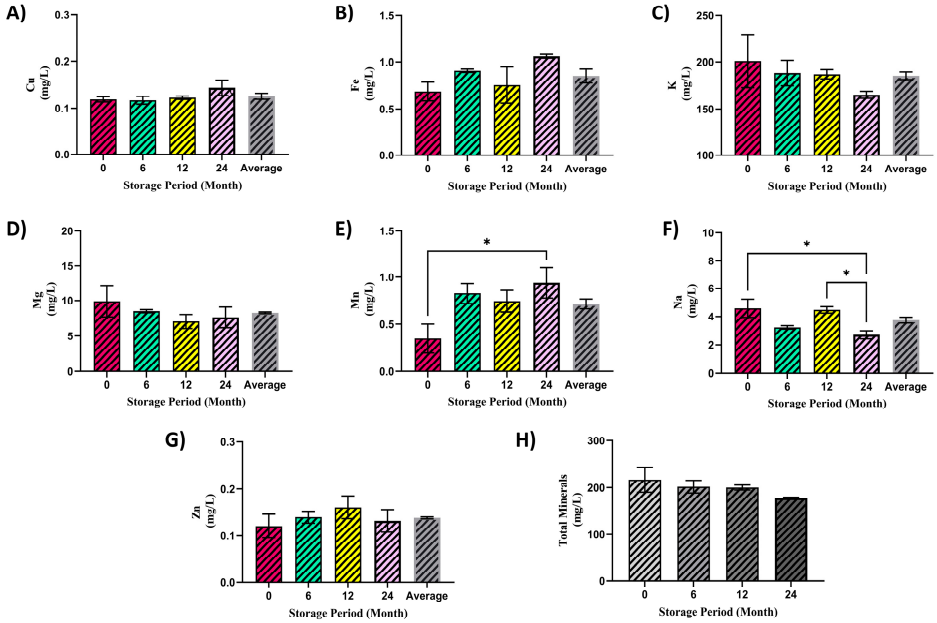

Figure 5. Cu (**A**), Fe (**B**), K (**C**), Mg (**D**), Mn (**E**), Na (**F**), Zn (**G**), and total mineral (**H**) of the ultrasound-treated watermelon traditional vinegar sample. Letters atop bars indicate statistically significant differences (ns: no significant; * $p < 0.05$; (n = 3 ± SD).

4. Conclusions

This study aimed to optimize the α-amylase inhibitory activity, ACE inhibitory activity, and α-glucosidase inhibitory values using the RSM modeling of different ultrasound treatments and to investigate the antioxidant activity, bioactive content (total phenolics and total flavonoids), mineral composition, and phenolic compounds after 24-month storage of optimized watermelon vinegar. The effects of the 24-month storage process on the DPPH, CUPRAC lycopene, TPC, and TAC values were significant. In this study, ultrasonic treatment was found to have a considerable effect on the antioxidant activity, bioactive content (total phenolics and flavonoids), mineral composition, and phenolic compounds of watermelon vinegar. With the help of RSM, critical process parameters can be optimized for the production of vinegar with good antidiabetic and antihypertensive effects. Fifteen phenolic compounds were detected in the ultrasound-treated watermelon vinegar. Our findings confirmed that UT-WV K, Mg, and Na were the main mineral compounds, and these compounds decreased during storage. For α-amylase inhibitory activity %, ACE inhibitory activity %, and α-glucosidase inhibitory activity %, the first-month values were significantly higher than those at the end of storage (24 months). The current study shows that fresh watermelon vinegar generally contains more bioactive compounds than the sample stored for 24 months. This study is the first to examine optimized watermelon vinegar, in addition to total phenolics and total flavonoids, the mineral composition, phenolic compounds, antioxidant activity, α-amylase inhibitory activity %, ACE inhibitory activity %, and α-glucosidase inhibitory activities of vinegar following 24 months of storage were determined. These results will pave the way for in vivo studies.

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References

1. Guo, Z.; Luo, C.X.; Wu, H.J.; Peng, B.; Kang, B.S.; Liu, L.M.; Zhang, M.; Gu, Q.S. Colletotrichum Species Associated with Anthracnose Disease of Watermelon (*Citrullus lanatus*) in China. *J. Fungi* **2022**, *8*, 790. [CrossRef]
2. Maletti, L.; D'Eusanio, V.; Durante, C.; Marchetti, A.; Tassi, L. VOCs Analysis of Three Different Cultivars of Watermelon (*Citrullus lanatus* L.) Whole Dietary Fiber. *Molecules* **2022**, *27*, 8747. [CrossRef]
3. Ijaz, A.; Tufail, T.; Saeed, F.; Afzaal, M.; Shahid, M.Z.; Suleria, H.A.R. Health Benefits of Watermelon (*Citrullus lanatus*). In *Bioactive Compounds from Multifarious Natural Foods for Human Health*; Apple Academic Press: Palm Bay, FL, USA, 2022; pp. 77–98. [CrossRef]
4. Wahid, M.; Saqib, F. Scientific Basis for Medicinal Use of *Citrullus lanatus* (Thunb.) in Diarrhea and Asthma: In Vitro, In Vivo and In Silico Studies. *Phytomedicine* **2022**, *98*, 153978. [CrossRef] [PubMed]
5. Öztürk, H.İ. Kardinal Üzümü, Napolyon Kirazı, Mürdüm Eriği, Kivi ve Şeftali Meyvelerinden Doğal Fermantasyonla Sirke Üretim Potansiyeli: Fizikokimyasal ve Duyusal Özellikler. *Akad. Gıda* **2022**, *20*, 54–62. [CrossRef]
6. Kandylis, P. Innovative Vinegar Products. In *Advances in Vinegar Production*; CRC Press: Boca Raton, FL, USA, 2019; pp. 265–297. [CrossRef]
7. Park, W.L.; Cho, H.D.; Kim, J.H.; Min, H.J.; Seo, K. Il Antioxidant Activity and Blood Alcohol Concentration Lowering Effect of Fermented Hovenia Dulcis Fruit Vinegar. *Food Sci. Biotechnol.* **2023**, *32*, 299–308. [CrossRef] [PubMed]
8. Kara, M.; Assouguem, A.; El Fadili, M.; Benmessaoud, S.; Alshawwa, S.Z.; Al Kamaly, O.; Saghrouchni, H.; Zerhouni, A.R.; Bahhou, J. Contribution to the Evaluation of Physicochemical Properties, Total Phenolic Content, Antioxidant Potential, and Antimicrobial Activity of Vinegar Commercialized in Morocco. *Molecules* **2022**, *27*, 770. [CrossRef] [PubMed]
9. Budak, H.N.; Guzel-Seydim, Z.B. Antioxidant Activity and Phenolic Content of Wine Vinegars Produced by Two Different Techniques. *J. Sci. Food Agric.* **2010**, *90*, 2021–2026. [CrossRef]
10. Bakir, S.; Toydemir, G.; Boyacioglu, D.; Beekwilder, J.; Capanoglu, E. Fruit Antioxidants during Vinegar Processing: Changes in Content and in Vitro Bio-Accessibility. *Int. J. Mol. Sci.* **2016**, *17*, 1658. [CrossRef]
11. Wang, Z.; Lin, K.; Wang, Y.; Wen, L. Study on Development of Watermelon Rind Fruit Vinegar. *China Condiment* **2012**, *7*, 58–61.
12. Chen, Y.; Bai, Y.; Li, D.; Wang, C.; Xu, N.; Hu, Y. Improvement of the Flavor and Quality of Watermelon Vinegar by High Ethanol Fermentation Using Ethanol-Tolerant Acetic Acid Bacteria. *Int. J. Food Eng.* **2017**, *13*, 20160222. [CrossRef]
13. Wu, Q.; Luo, Q.; Xiao, J.; Tang, S.; Chen, Y.; Shen, Y.; Xu, N.; Zhou, M.; Hu, Y.; Wang, C.; et al. Catechin-Iron as a New Inhibitor to Control Advanced Glycation End-Products Formation during Vinegar Storage. *LWT* **2019**, *112*, 108245. [CrossRef]
14. Feng, N.; Feng, Y.; Zhang, F.; Yan, J.; Niu, M.; Shi, L.; Xiong, H.; Zhou, M.; Wu, Q. Inhibition Mechanism of Mango Vinegar on Protein-AGEs Digestion in Vitro Gastrointestinal Environment. *LWT* **2023**, *186*, 115246. [CrossRef]
15. Cavdaroglu, C.; Ozen, B. Detection of Vinegar Adulteration with Spirit Vinegar and Acetic Acid Using UV–Visible and Fourier Transform Infrared Spectroscopy. *Food Chem.* **2022**, *379*, 132150. [CrossRef] [PubMed]
16. Kumar, N.; Kumar, G.; Prabhakar, P.K.; Sahu, J.K.; Naik, S. Ultrasound-Assisted Extraction of Bioactive Compounds from Giloy (*Tinospora cordifolia*) Stem: Quantitative Process Optimization and Bioactives Analysis. *J. Food Process Eng.* **2023**, *46*, e14259. [CrossRef]
17. Seong, E.; Heo, H.; Sang Jeong, H.; Lee, H.; Lee, J. Enhancement of Bioactive Compounds and Biological Activities of Centella Asiatica through Ultrasound Treatment. *Ultrason. Sonochem.* **2023**, *94*, 106353. [CrossRef]
18. Aghajanzadeh, S.; Ziaiifar, A.M. Pasteurization of Juices with Non-Thermal Technologies. In *Sustainable Food Processing and Engineering Challenges*; Academic Press: Cambridge, MA, USA, 2021; pp. 25–73. [CrossRef]
19. Gavahian, M.; Manyatsi, T.S.; Morata, A.; Tiwari, B.K. Ultrasound-Assisted Production of Alcoholic Beverages: From Fermentation and Sterilization to Extraction and Aging. *Compr. Rev. Food Sci. Food Saf.* **2022**, *21*, 5243–5271. [CrossRef]
20. Beitia, E.; Gkogka, E.; Chanos, P.; Hertel, C.; Heinz, V.; Valdramidis, V.; Aganovic, K. Microbial Decontamination Assisted by Ultrasound-Based Processing Technologies in Food and Model Systems: A Review. *Compr. Rev. Food Sci. Food Saf.* **2023**, *22*, 2802–2849. [CrossRef]
21. Gueffai, A.; Gonzalez-Serrano, D.J.; Christodoulou, M.C.; Orellana-Palacios, J.C.; Ortega, M.L.S.; Ouldmoumna, A.; Kiari, F.Z.; Ioannou, G.D.; Kapnissi-Christodoulou, C.P.; Moreno, A.; et al. Phenolics from Defatted Black Cumin Seeds (*Nigella sativa* L.): Ultrasound-Assisted Extraction Optimization, Comparison, and Antioxidant Activity. *Biomolecules* **2022**, *12*, 1311. [CrossRef]
22. Ribeiro, D.N.; Alves, F.M.S.; dos Santos Ramos, V.H.; Alves, P.; Narain, N.; Vedoy, D.R.L.; Cardozo-Filho, L.; de Jesus, E. Extraction of Passion Fruit (*Passiflora cincinnata* Mast.) Pulp Oil Using Pressurized Ethanol and Ultrasound: Antioxidant Activity and Kinetics. *J. Supercrit. Fluids* **2020**, *165*, 104944. [CrossRef]

23. Rashid, R.; Mohd Wani, S.; Manzoor, S.; Masoodi, F.A.; Masarat Dar, M. Green Extraction of Bioactive Compounds from Apple Pomace by Ultrasound Assisted Natural Deep Eutectic Solvent Extraction: Optimisation, Comparison and Bioactivity. *Food Chem.* **2023**, *398*, 133871. [CrossRef]
24. Rigane, G.; Yahyaoui, A.; Acar, A.; Mnif, S.; Salem, R.B.; Arslan, D. Change in Some Quality Parameters and Oxidative Stability of Olive Oils with Regard to Ultrasound Pretreatment, Depitting and Water Addition. *Biotechnol. Rep.* **2020**, *26*, e00442. [CrossRef]
25. Shekhar, S.; Prakash, P.; Singha, P.; Prasad, K.; Singh, S.K. Modeling and Optimization of Ultrasound-Assisted Extraction of Bioactive Compounds from Allium Sativum Leaves Using Response Surface Methodology and Artificial Neural Network Coupled with Genetic Algorithm. *Foods* **2023**, *12*, 1925. [CrossRef]
26. Yıkmış, S. Optimization of Uruset Apple Vinegar Production Using Response Surface Methodology for the Enhanced Extraction of Bioactive Substances. *Foods* **2019**, *8*, 107. [CrossRef]
27. Siddeeg, A.; Zeng, X.A.; Rahaman, A.; Manzoor, M.F.; Ahmed, Z.; Ammar, A.F. Quality Characteristics of the Processed Dates Vinegar under Influence of Ultrasound and Pulsed Electric Field Treatments. *J. Food Sci. Technol.* **2019**, *56*, 4380–4389. [CrossRef] [PubMed]
28. Zia, S.; Khan, M.R.; Shabbir, M.A.; Aadil, R.M. An Update on Functional, Nutraceutical and Industrial Applications of Watermelon by-Products: A Comprehensive Review. *Trends Food Sci. Technol.* **2021**, *114*, 275–291. [CrossRef]
29. Ajiboye, B.O.; Shonibare, M.T.; Oyinloye, B.E. Antidiabetic Activity of Watermelon (*Citrullus lanatus*) Juice in Alloxan-Induced Diabetic Rats. *J. Diabetes Metab. Disord.* **2020**, *19*, 343–352. [CrossRef]
30. Liljeberg, H.; Björck, I. Delayed Gastric Emptying Rate May Explain Improved Glycaemia in Healthy Subjects to a Starchy Meal with Added Vinegar. *Eur. J. Clin. Nutr.* **1998**, *52*, 368–371. [CrossRef] [PubMed]
31. Brighenti, F.; Castellani, G.; Benini, L.; Casiraghi, M.C.; Leopardi, E.; Crovetti, R.; Testolin, G. Effect of Neutralized and Native Vinegar on Blood Glucose and Acetate Responses to a Mixed Meal in Healthy Subjects. *Eur. J. Clin. Nutr.* **1995**, *49*, 242–247.
32. Sajjad, S.; Israr, B.; Ali, F.; Pasha, I. Investigating The Effect Of Phytochemicals Rich Watermelon Seeds against Hypertension. *Pak. J. Agric. Sci.* **2020**, *56*, 1157–1164.
33. Mazloomi, S.N.; Mora, L.; Aristoy, M.C.; Mahoonak, A.S.; Ghorbani, M.; Houshmand, G.; Toldrá, F. Impact of Simulated Gastrointestinal Digestion on the Biological Activity of an Alcalase Hydrolysate of Orange Seed (Siavaraze, *Citrus sinensis*) by-Products. *Foods* **2020**, *9*, 1217. [CrossRef]
34. Kim, S.H.; Jeong, W.S.; Kim, S.Y.; Yeo, S.H. Quality and Functional Characterization of Acetic Acid Bacteria Isolated from Farm-Produced Fruit Vinegars. *Fermentation* **2023**, *9*, 447. [CrossRef]
35. Morgan, J.; Mosawy, S. The Potential of Apple Cider Vinegar in the Management of Type 2 Diabetes. *Int. J. Diabetes Res.* **2016**, *2016*, 129–134. [CrossRef]
36. Volino-Souza, M.; Oliveira, G.V.D.; Conte-Junior, C.A.; Figueroa, A.; Alvares, T.S. Current Evidence of Watermelon (*Citrullus lanatus*) Ingestion on Vascular Health: A Food Science and Technology Perspective. *Nutrients* **2022**, *14*, 2913. [CrossRef]
37. Saqib, F.; Wahid, M.; AL-Huqail, A.A.; Ahmedah, H.T.; Bigiu, N.; Irimie, M.; Moga, M.; Marc (Vlaic), R.A.; Pop, O.L.; Chicea, L.M. Metabolomics Based Mechanistic Insights to Vasorelaxant and Cardioprotective Effect of Ethanolic Extract of *Citrullus lanatus* (Thunb.) Matsum. & Nakai. Seeds in Isoproterenol Induced Myocardial Infraction. *Phytomedicine* **2022**, *100*, 154069. [CrossRef] [PubMed]
38. Cushman, D.W.; Cheung, H.S. Spectrophotometric Assay and Properties of the Angiotensin-Converting Enzyme of Rabbit Lung. *Biochem. Pharmacol.* **1971**, *20*, 1637–1648. [CrossRef]
39. Zhang, L.; Hogan, S.; Li, J.; Sun, S.; Canning, C.; Zheng, S.J.; Zhou, K. Grape Skin Extract Inhibits Mammalian Intestinal α-Glucosidase Activity and Suppresses Postprandial Glycemic Response in Streptozocin-Treated Mice. *Food Chem.* **2011**, *126*, 466–471. [CrossRef]
40. Oms-Oliu, G.; Odriozola-Serrano, I.; Soliva-Fortuny, R.; Martín-Belloso, O. Effects of High-Intensity Pulsed Electric Field Processing Conditions on Lycopene, Vitamin C and Antioxidant Capacity of Watermelon Juice. *Food Chem.* **2009**, *115*, 1312–1319. [CrossRef]
41. Singleton, V.; Rossi, A. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagent. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158. [CrossRef]
42. Zhishen, J.; Mengcheng, T.; Jianming, W. The Determination of Flavonoid Contents in Mulberry and Their Scavenging Effects on Superoxide Radicals. *Food Chem.* **1999**, *64*, 555–559. [CrossRef]
43. Apak, R.; Güçlü, K.; Özyürek, M.; Esin Karademir, S.; Erçağ, E. The Cupric Ion Reducing Antioxidant Capacity and Polyphenolic Content of Some Herbal Teas. *Int. J. Food Sci. Nutr.* **2006**, *57*, 292–304. [CrossRef] [PubMed]
44. Grajeda-Iglesias, C.; Salas, E.; Barouh, N.; Baréa, B.; Panya, A.; Figueroa-Espinoza, M.C. Antioxidant Activity of Protocatechuates Evaluated by DPPH, ORAC, and CAT Methods. *Food Chem.* **2016**, *194*, 749–757. [CrossRef] [PubMed]
45. Giusti, M.M.; Wrolstad, R.E. Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. *Curr. Protoc. Food Anal. Chem.* **2001**, F1.2.1–F1.2.13. [CrossRef]
46. Cemeroğlu, B. *Gıda Analizleri*, 2nd, ed.; Nobel Yayıncılık: Ankara, Turkey, 2010; ISBN 9759857868.
47. Portu, J.; López, R.; Santamaría, P.; Garde-Cerdán, T. Elicitation with Methyl Jasmonate Supported by Precursor Feeding with Phenylalanine: Effect on Garnacha Grape Phenolic Content. *Food Chem.* **2017**, *237*, 416–422. [CrossRef]
48. Sezer, B.; Apaydin, H.; Bilge, G.; Boyaci, I.H. Detection of Pistacia Vera Adulteration by Using Laser Induced Breakdown Spectroscopy. *J. Sci. Food Agric.* **2019**, *99*, 2236–2242. [CrossRef] [PubMed]

49. Santos, H.O.; de Moraes, W.M.A.M.; da Silva, G.A.R.; Prestes, J.; Schoenfeld, B.J. Vinegar (Acetic Acid) Intake on Glucose Metabolism: A Narrative Review. *Clin. Nutr. ESPEN* **2019**, *32*, 1–7. [CrossRef]
50. Kondo, S.; Tayama, K.; Tsukamoto, Y.; Ikeda, K.; Yamori, Y. Antihypertensive Effects of Acetic Acid and Vinegar on Spontaneously Hypertensive Rats. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 2690–2694. [CrossRef]
51. Yıkmış, S.; Aksu, F.; Altunatmaz, S.S.; Çöl, B.G. Ultrasound Processing of Vinegar: Modelling the Impact on Bioactives and Other Quality Factors. *Foods* **2021**, *10*, 1703. [CrossRef]
52. Erdal, B.; Akalın, R.B.; Yılmaz, B.; Bozgeyik, E.; Yıkmış, S. Application of Ultrasound to the Organic Cornelian Cherry (*Cornus mas* L.) Vinegar: Changes in Antibacterial, Antidiabetic, Antihypertensive, and Anticancer Activities. *J. Food Process Preserv.* **2022**, *46*, e16952. [CrossRef]
53. Yıkmış, S.; Ozer, H.; Levent, O.; Çöl, B.G.; Erdal, B. Effect of Thermosonication and Thermal Treatments on Antidiabetic, Antihypertensive, Mineral Elements and in Vitro Bioaccessibility of Bioactive Compounds in Freshly Squeezed Pomegranate Juice. *J. Food Meas. Charact.* **2022**, *16*, 3023–3041. [CrossRef]
54. Xue, R.; Cui, E.-L.; Hu, G.-Q.; Zhu, M.-Q. The Composition, Physicochemical Properties, Antimicrobial and Antioxidant Activity of Wood Vinegar Prepared by Pyrolysis of Eucommia Ulmoides Oliver Branches under Different Refining Methods and Storage Conditions. *Ind. Crops Prod.* **2022**, *178*, 114586. [CrossRef]
55. Muzaffar, S.; Ahmad, M.; Wani, S.M.; Gani, A.; Baba, W.N.; Shah, U.; Khan, A.A.; Masoodi, F.A.; Gani, A.; Wani, T.A. Ultrasound Treatment: Effect on Physicochemical, Microbial and Antioxidant Properties of Cherry (*Prunus avium*). *J. Food Sci. Technol.* **2016**, *53*, 2752–2759. [CrossRef]
56. Wang, F.; Song, Y.; Vidyarthi, S.K.; Zhang, R. Physicochemical Properties, and Volatile Compounds of Blackened Jujube Vinegar as Prepared by Optimized Fermentation Process. *Int. J. Food Prop.* **2022**, *25*, 288–304. [CrossRef]
57. Oliveira, C.S.; Gomes, F.S.; Constant, L.S.; Silva, L.F.M.; Godoy, R.L.O.; Tonon, R.V.; Cabral, L.M.C. Integrated Membrane Separation Processes Aiming to Concentrate and Purify Lycopene from Watermelon Juice. *Innov. Food Sci. Emerg. Technol.* **2016**, *38*, 149–154. [CrossRef]
58. Makroo, H.A.; Saxena, J.; Rastogi, N.K.; Srivastava, B. Ohmic Heating Assisted Polyphenol Oxidase Inactivation of Watermelon Juice: Effects of the Treatment on PH, Lycopene, Total Phenolic Content, and Color of the Juice. *J. Food Process Preserv.* **2017**, *41*, e13271. [CrossRef]
59. Quek, S.Y.; Chok, N.K.; Swedlund, P. The Physicochemical Properties of Spray-Dried Watermelon Powders. *Chem. Eng. Process. Process Intensif.* **2007**, *46*, 386–392. [CrossRef]
60. Acharya, P.; Singh, J.; Jayaprakasha, G.K.; Jifon, J.L.; Crosby, K.M.; Patil, B.S. Impact of Storage Period and Nanoparticle Treatment on Phytochemical Composition of Watermelons (*Citrullus lanatus*). *J. Food Compos. Anal.* **2021**, *104*, 104139. [CrossRef]
61. Fish, W.W.; Davis, A.R. The Effects of Frozen Storage Conditions on Lycopene Stability in Watermelon Tissue. *J. Agric. Food Chem.* **2003**, *51*, 3582–3585. [CrossRef]
62. Perkins-Veazie, P.; Collins, J.K. Flesh Quality and Lycopene Stability of Fresh-Cut Watermelon. *Postharvest Biol. Technol.* **2004**, *31*, 159–166. [CrossRef]
63. Galdeano, M.C.; dos Santos Gomes, F.; Chávez, D.W.H.; Almeida, E.L.; Moulin, L.C.; de Grandi Castro Freitas de Sá, D.; Tonon, R.V. Lycopene-Rich Watermelon Concentrate Used as a Natural Food Colorant: Stability during Processing and Storage. *Food Res. Int.* **2022**, *160*, 111691. [CrossRef]
64. Kyriacou, M.C.; Soteriou, G. Quality and Postharvest Performance of Watermelon Fruit in Response to Grafting on Interspecific Cucurbit Rootstocks. *J. Food Qual.* **2015**, *38*, 21–29. [CrossRef]
65. Perkins-Veazie, P.; Collins, J.K.; Pair, S.D.; Roberts, W. Lycopene Content Differs among Red-Fleshed Watermelon Cultivars. *J. Sci. Food Agric.* **2001**, *81*, 983–987. [CrossRef]
66. Ali, M.; Manzoor, M.F.; Goksen, G.; Aadil, R.M.; Zeng, X.A.; Iqbal, M.W.; Lorenzo, J.M. High-Intensity Ultrasonication Impact on the Chlorothalonil Fungicide and Its Reduction Pathway in Spinach Juice. *Ultrason. Sonochem.* **2023**, *94*, 106303. [CrossRef]
67. Rybak, M.; Wojdyło, A. Inhibition of α-Amylase, α-Glucosidase, Pancreatic Lipase, 15-Lipooxygenase and Acetylcholinesterase Modulated by Polyphenolic Compounds, Organic Acids, and Carbohydrates of Prunus Domestica Fruit. *Antioxidants* **2023**, *12*, 1380. [CrossRef]
68. Choi, M.H.; Kim, G.H.; Lee, H.S. Effects of Ascorbic Acid Retention on Juice Color and Pigment Stability in Blood Orange (*Citrus sinensis*) Juice during Refrigerated Storage. *Food Res. Int.* **2002**, *35*, 753–759. [CrossRef]
69. Montenegro-Landívar, M.F.; Tapia-Quirós, P.; Vecino, X.; Reig, M.; Valderrama, C.; Granados, M.; Cortina, J.L.; Saurina, J. Polyphenols and Their Potential Role to Fight Viral Diseases: An Overview. *Sci. Total Environ.* **2021**, *801*, 149719. [CrossRef]
70. Duan, W.; Xia, T.; Zhang, B.; Li, S.; Zhang, C.; Zhao, C.; Song, J.; Wang, M. Changes of Physicochemical, Bioactive Compounds and Antioxidant Capacity during the Brewing Process of Zhenjiang Aromatic Vinegar. *Molecules* **2019**, *24*, 3935. [CrossRef] [PubMed]
71. Rawson, A.; Tiwari, B.K.; Patras, A.; Brunton, N.; Brennan, C.; Cullen, P.J.; O'Donnell, C. Effect of Thermosonication on Bioactive Compounds in Watermelon Juice. *Food Res. Int.* **2011**, *44*, 1168–1173. [CrossRef]
72. Chen, G.L.; Zheng, F.J.; Lin, B.; Lao, S.B.; He, J.; Huang, Z.; Zeng, Y.; Sun, J.; Verma, K.K. Phenolic and Volatile Compounds in the Production of Sugarcane Vinegar. *ACS Omega* **2020**, *5*, 30587–30595. [CrossRef]

73. Chatatikun, M.; Tedasen, A.; Pattaranggoon, N.C.; Palachum, W.; Chuaijit, S.; Mudpan, A.; Pruksaphanrat, S.; Sohbenalee, S.; Yamasaki, K.; Klangbud, W.K. Antioxidant Activity, Anti-Tyrosinase Activity, Molecular Docking Studies, and Molecular Dynamic Simulation of Active Compounds Found in Nipa Palm Vinegar. *PeerJ* **2023**, *11*, e16494. [CrossRef]
74. Wu, Q.; Kong, Y.; Liang, Y.; Niu, M.; Feng, N.; Zhang, C.; Qi, Y.; Guo, Z.; Xiao, J.; Zhou, M.; et al. Protective Mechanism of Fruit Vinegar Polyphenols against AGEs-Induced Caco-2 Cell Damage. *Food Chem. X* **2023**, *19*, 100736. [CrossRef]
75. Tokatlı Demirok, N.; Yıkmış, S.; Duman Altan, A.; Apaydın, H. Optimization of Ultrasound-Treated Horsetail-Fortified Traditional Apple Vinegar Using RSM and ANFIS Modeling: Bioactive and Sensory Properties. *J. Food Meas. Charact.* **2023**, *18*, 256–271. [CrossRef]
76. Liu, Q.; Tang, G.Y.; Zhao, C.N.; Gan, R.Y.; Li, H. Bin Antioxidant Activities, Phenolic Profiles, and Organic Acid Contents of Fruit Vinegars. *Antioxidants* **2019**, *8*, 78. [CrossRef] [PubMed]
77. Wen, M.; Zhou, F.; Zhu, M.; Han, Z.; Lai, G.; Jiang, Z.; Long, P.; Zhang, L. Monitoring of Pickled Tea during Processing: From LC-MS Based Metabolomics Analysis to Inhibitory Activities on α-Amylase and α-Glycosidase. *J. Food Compos. Anal.* **2023**, *117*, 105108. [CrossRef]
78. Yim, E.-J.; Jo, S.-W.; Lee, E.-S.; Park, H.-S.; Ryu, M.-S.; Uhm, T.-B.; Kim, H.-Y.; Cho, S.-H. Fermentation Characteristics of Mulberry (*Cudrania tricuspidata*) Fruit Vinegar Produced by Acetic Acid Bacteria Isolated from Traditional Fermented Foods. *Korean J. Food Preserv.* **2015**, *22*, 108–118. [CrossRef]
79. Rasouli, H.; Hosseini-Ghazvini, S.M.B.; Adibi, H.; Khodarahmi, R. Differential α-Amylase/α-Glucosidase Inhibitory Activities of Plant-Derived Phenolic Compounds: A Virtual Screening Perspective for the Treatment of Obesity and Diabetes. *Food Funct.* **2017**, *8*, 1942–1954. [CrossRef] [PubMed]
80. National Agricultural Library. Available online: https://www.nal.usda.gov/ (accessed on 17 October 2023).
81. Ozturk, I.; Caliskan, O.; Tornuk, F.; Ozcan, N.; Yalcin, H.; Baslar, M.; Sagdic, O. Antioxidant, Antimicrobial, Mineral, Volatile, Physicochemical and Microbiological Characteristics of Traditional Home-Made Turkish Vinegars. *LWT Food Sci. Technol.* **2015**, *63*, 144–151. [CrossRef]
82. Antoniewicz, J.; Jakubczyk, K.; Kupnicka, P.; Bosiacki, M.; Chlubek, D.; Janda, K. Analysis of Selected Minerals in Homemade Grape Vinegars Obtained by Spontaneous Fermentation. *Biol. Trace Elem. Res.* **2022**, *200*, 910–919. [CrossRef]
83. Chou, C.H.; Liu, C.W.; Yang, D.J.; Wu, Y.H.S.; Chen, Y.C. Amino Acid, Mineral, and Polyphenolic Profiles of Black Vinegar, and Its Lipid Lowering and Antioxidant Effects in Vivo. *Food Chem.* **2015**, *168*, 63–69. [CrossRef]