J Food Sci Technol (September 2020) 57(9):3445–3456
https://doi.org/10.1007/s13197-020-04379-5

ORIGINAL ARTICLE

Ultrasound processing of verjuice (unripe grape juice) vinegar: effect on bioactive compounds, sensory properties, microbiological quality and anticarcinogenic activity

Seydi Yıkmış[1] · Esra Bozgeyik[2] · Mehmet Ali Şimşek[3]

Revised: 4 March 2020 / Accepted: 27 March 2020 / Published online: 4 April 2020

Abstract Verjuice is one of the alternative fruit juices recently obtained from unripe grapes. In this study, the aim was primarily to optimize the process conditions for the enrichment of bioactive components in verjuice vinegar with ultrasound treatment. For this purpose, ultrasound treatment was applied to vinegar samples at different times (2, 4, 6, 8 and 10 min), different amplitudes (60%, 65%, 70%, 75% and 80%) and 26 kHz frequency. Total phenolic content (TPC), total flavonoid content (TFC), total antioxidant capacity (1,1-diphenyl-2-picrylhydrazyl (DPPH) and cupric reducing antioxidant capacity (CUPRAC) were evaluated for optimization (response surface methodology (RSM) and genetic algorithm (GA)) of process conditions. The sensory properties, microbiological quality and anticarcinogenic activity were then evaluated for the ultrasound-treated verjuice vinegar (UVV) (9.4 min and 68.7 amplitude result of RSM), traditional verjuice vinegar and pasteurized verjuice vinegar samples obtained from the optimization. At the end of the RSM optimization, CUPRAC (464.44 mg TEAC/mL), DPPH (0.694 mg TEAC/mL), TFC (70.85 mg CE/mL) and TPC (12.22 mg GAE/mL) were determined. RSM and GA results were found to be approximately the same. Analysis results showed that ultrasound-treated verjuice vinegar was enriched bioactive components compared to other samples. Verjuice vinegar showed anticarcinogenic effects. The UVV sample was generally appreciated in sensory evaluation. As a result, ultrasound treatment of verjuice vinegar was found to be successful.

Keywords Ultrasound · Verjuice vinegar · Response surface methodology · Genetic algorithm · Anticarcinogenic

Introduction

Vinegar is a special product produced from various raw materials containing carbohydrates through yeasts and acetic acid bacteria. Vinegar production involves the conversion of fermentable sugars by yeast into ethanol under anaerobic conditions and the second step involves the production of acetic acid by acetic acid bacteria (AAB) under aerobic conditions using ethanol present in the environment. The conversion of ethanol to acetic acid in vinegar production takes place through two biochemical reactions. During these reactions, acetaldehyde is produced primarily through oxidation of ethanol by the alcohol dehydrogenase enzyme, the resulting acetaldehyde is then converted to acetic acid by the aldehyde dehydrogenase enzyme (Gullo et al. 2014).

Vinegar, which is widely used as a flavoring and preserving agent in foods, has also been used in the treatment of various diseases since ancient times. It has been used traditionally. Thanks to various phenolic compounds, amino acids, vitamins, organic acids, antioxidants and melanoidins contained in vinegar, it has many beneficial effects on health including antimicrobial effects, reducing

✉ Seydi Yıkmış
syikmis@nku.edu.tr

[1] Department of Nutrition and Dietetics, School of Health Sciences, Tekirdağ Namık Kemal University, Tekirdağ, Turkey

[2] Department of Medical Biology, Faculty of Medicine, Tekirdag Namik Kemal University, Tekirdağ, Turkey

[3] Department of Computer Technologies, Vocational School of Technical Sciences, Tekirdag Namik Kemal University, Tekirdağ, Turkey

cardiovascular diseases, slowing gastric emptying and providing a feeling of satiety, antidiabetic, anticarcinogenic, antitumor and anti-infectious effects (Chen et al. 2016; Karabiyikli and Sengun 2017).

Today, due to the awareness of society about nutrition, the interest in functional foods is increasing. In recent years, it was observed that the consumption of vinegar, which attracts attention due to its effects on health, has increased. It is reported that the methods used during the production of vinegar affect bioactive components in vinegar, and that the vinegar produced by traditional methods may have more functional properties than industrial vinegar (Pazuch et al. 2015; Karabiyikli and Sengun 2017). Phenolic substances, which vary depending on the raw material used and the production method, affect the antioxidant and antimicrobial potential of vinegar (Karabiyikli and Sengun 2017). In most studies, a limited number of vinegar varieties have been examined; however, a limited number of studies have examined the characteristics of different vinegars produced traditionally (Chen et al. 2016).

The increase in the popularity of fresh or natural products has led to an increase in study about natural processing. Thermal pasteurization is a technology used to provide microbial inactivation and prolong the shelf life of products. However, it has negative effects on nutritional and physicochemical parameters (Rawson et al. 2011). Increasing interest in products that have undergone minimal changes due to their natural structure has supported the development of innovative non-thermal food preservation methods. One of the most commonly used methods is ultrasound technology. Ultrasound is defined as mechanical sound waves at frequencies outside the sound range that can be heard by the human ear (Chemat and Khan 2011). Ultrasound treatment, the improvement of bioactive compounds by various processes alternative is affordable and efficient, and reproducible method (Roselló-Soto et al. 2015b). Ultrasound has been identified as a potential technology by the US Food and Drug Administration (FDA) to meet the need for 5 log reductions in related microorganisms found in fruit juices (Salleh-Mack and Roberts 2007). Research has shown that heat treatment is also a good alternative and has minimal effect on quality values (Mason et al. 2003; Rojas et al. 2017). The mechanism of ultrasound treatment was explained mainly by two events. In the physical event, microjets and shock waves are the result of acoustic cavitation. In the chemical event, it was stated that precipitation forming as a result of cavitation may lead to the formation of free radicals from the sonolysis of water vapor (Zoran et al. 2012; Gao et al. 2014). In the research reports, carrot-grapes (Nadeem et al. 2018), apples (Abid et al. 2014), Kasturi lime (Bhat et al. 2011), cranberry (Jambrak et al. 2017), orange (Samani et al. 2015) and mosambi (Siwach and KumarKumar 2012) juice quality and nutritional values were found to have the least losses.

Optimization is used to make process designs more efficient (improving bioactive components in the most efficient way). Artificial intelligence optimization techniques are used for problems that cannot be solved by classical methods. One of these techniques is the genetic algorithm (GA). GA is a random search and optimization technique based on the natural genetic principle. The main purpose of GA is to find a realistic answer to problems without a polynomial solution (Deb 2012). In this study, the aim was to perform ultrasound treatment on traditionally produced and scientifically researched verjuice vinegar and to optimize bioactive components (total phenolic content, total flavonoid content, DPPH and CUPRAC) using surface response method and genetic algorithm. At the same time, the general microbiology, sensory properties and anticarcinogenic effects of pasteurized vinegar, traditional vinegar and ultrasound-treated vinegar were compared.

Materials and methods

Vinegar production

The traditional method was used for the production of vinegar. Research unripe grape was the raw material for the production of vinegar supplied from Tekirdağ, Turkey with verjuice of 5 kg used for each experiment. The ripe fruits were pressed and filtered to produce juice. *Saccharomyces cerevisiae* (3%) was inoculated into sterile jars for ethanol fermentation. The fermentation caps were closed and alcohol was allowed to ferment for 30 days at 25 °C. This wine was transferred into the sterile jars and inoculated with concentration of the vinegar (10%) as a natural acetic acid culture. The mixture was regularly ventilated during acetic acid fermentation. The mixture was fermented for 60 days at 28 °C until the ethanol content was 0.5–1%. Periodic acidity measurements were made and mothers of vinegar were formed on the surface of the vinegar at the end of fermentation. Vinegar samples were stored at 4 °C in 100 ml sterile glass jars for use in analysis. The control (TVV) sample was untreated traditional verjuice vinegar. Pasteurized verjuice vinegar (PVV) was processed at 65 °C for 30 min. Based on the results of optimization conditions, ultrasound-treated verjuice vinegar (UVV) was obtained. Tests were performed in triplicate.

Ultrasound treatments

Sonication treatments were performed directly after extraction of fresh juice. The verjuice vinegar was treated with 26 kHz frequency, at different times (2, 4, 6, 8 and 10 min) and amplitudes (40%, 50%, 60%, 70% and 80%). The sonication was performed at 26 kHz frequency and temperature of 20 °C using a 200 W ultrasonic processor (Model UP200St, Germany). All the sonication treatments were carried out in the dark to avoid any possible interference from light. Verjuice vinegar samples (sonicated) were kept in sterilized and air tight media bottles, and stored at 4 °C until further analysis.

Experimental design

Response surface modeling

The Response Surface Method (RSM) was used to understand the effect of ultrasound treatments on the quality parameters of the verjuice vinegar. Central Composite Design was chosen as the experimental design and a five-level, two-factor experimental design was created. There are 13 trial points for optimization (Table 1). Model adequacy, R^2 and corrected $-R^2$ coefficients, lack-of-fit tests and ANOVA results were evaluated. The independent variables were duration (X_1) and amplitude (X_2). Dependent variables were TPC, TFC, and total antioxidants (DPPH and CUPRAC).

Data analysis and optimization

Using the response surface methodology in MINTAB statistical software (Minitab 18.1, Minitab, Inc, USA, 2017), the second order-polynomial equation shown in the following equation was used to form the model equations:

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

In the equation, Y is the dependent variable, β_0 is the intersection term, β_i is the first order (linear) coefficient of equation, β_{ii} is the second order equation coefficient, β_{ij} is the two-factor cross-interaction coefficient, and X_i and X_j are independent variables. Three-dimensional response surface plots were developed using SigmaPlot 12.0 Statistical Analysis Software (Systat Software, Inc., San Jose, California, USA) and represent a function of two independent variables.

Modeling of genetic algorithm

While solving optimization problems using genetic algorithms (GA), populations of possible solutions were randomly selected. Each individual in the population is called a chromosome. To determine the success of chromosomes to solve the problem, the suitability value was determined. Four mathematical models for TPC, TFC, and total antioxidants (DPPH and CUPRAC) in RSM results were used as the objective function to calculate the suitability values. GA was applied separately for each objective function.

Selection of chromosome

In this study, the population size was determined as N = 20. The first population was formed by using the randomness principle according to the range of temperature and amplitude variables. In this study the roulette wheel, one of the commonly used parental selection techniques, was used as selection operator in this study (Deb 2012). The survival probability (*Pi*) of each chromosome is calculated as shown in Eq. (2). Pi is the probability of survival of each chromosome in each population. Fi is the fitness function value of the chromosome. In the roulette wheel method, the objective function values of all chromosomes found in the population are summed ($\sum Fj$). The cumulative probability values (Ki) for each chromosome are then calculated as shown in Eq. (3). For each chromosome, a random r number ($r \in [0, 1]$) was generated between 0 and 1. If $r < Ki$, chromosome i survives. In addition, elitiz was used to transfer the best individuals to the next population.

$$Pi = \frac{Fi}{\sum_{j=1}^{N} Fj} \qquad (2)$$

$$Ki = \sum_{j=1}^{i} Pi \qquad (3)$$

Genetic operators

After a certain number of iterations, more suitable chromosomes cannot be obtained in the population. Diversity of the population was determined by crossing and mutation operators and more suitable chromosomes were obtained.

The crossover probability (Pc) was determined before the main chromosomes were held to cross. In this study, Pc = 0.7 was determined and a single point crossover was performed. Two random chromosomes were identified from the population. A random number r ($r \in [0,1]$) is generated between 0 and 1. If $r < Pc$, the two chromosomes identified were crossed.

Table 1 Measured responses used in experimental design for RSM

Sample[a]	Encoded independent variables		Dependent variables			
	Time (X_1)	Amplitude (X_2)	Response 1 Total phenolics compound (mg GAE/L)	Response 2 Total flavonoids (mg CE/L)	Response 3 DPPH (mg TEAC/mL)	Response 4 CUPRAC (mg TEAC/mL)
PVV			384.77	59.54	0.629	0.758
TVV			447.44	64.42	0.674	0.795
1	8 (+ 1)	50 (− 1)	463.66	69.88	0.682	0.816
2	8 (+ 1)	70 (+ 1)	465.15	70.58	0.694	0.837
3	6 (0)	60 (0)	459.56	69.63	0.683	0.819
4	2 (− 1.41)	60 (0)	450.87	68.54	0.664	0.810
5	6 (0)	60 (0)	461.22	69.47	0.685	0.820
6	6 (0)	60 (0)	460.85	69.72	0.684	0.819
7	6 (0)	60 (0)	461.34	69.56	0.684	0.818
8	10 (+ 1.41)	60 (0)	464.74	70.68	0.683	0.835
9	4 (− 1)	70 (+ 1)	459.39	69.32	0.672	0.808
10	6 (0)	80 (+ 1.41)	464.64	70.36	0.688	0.827
11	4 (− 1)	50 (− 1)	450.71	68.17	0.681	0.817
12	6 (0)	60 (0)	460.13	69.44	0.685	0.819
13	6 (0)	40 (− 1.41)	455.67	68.04	0.684	0.815
Multiple response prediction/UVV	9.4 min	68.7 amplitude	464.44	70.85	0.694	0.846
R^2			97.67%	97.54%	99.09%	99.37%
Adj R^2			96.00%	95.79%	98.44%	98.92%
Pred R^2			83.50%	80.51%	94.64%	96.42%

PVV pasteurized verjuice vinegar; *TVV* traditional verjuice vinegar; *UVV* ultrasound-treated verjuice vinegar; *GAE* Gallic acid equivalent; *DDPH* radical scavenging activity; *CUPRAC* cupric reducing antioxidant capacity

The mutation operator was applied to prevent chromosomes from attaching to local minimum and maximum values. This was determined by the mutation probability (Pm). In this study, Pm = 0.05 was determined. A r number ($r \in [0,1]$) between 0 and 1 was determined for each chromosome in the population. If $r < Pm$, the corresponding chromosome mutates.

Iteration

Each step in which GA codes work is referred to as an iteration. At each step, natural selection, crossover and mutation operators act and a new population is formed. The suitability values of each chromosome in the newly formed population are compared to the chromosome with the best fit depth to date. This process ends with a stop criterion. The number of iterations for this software is 500.

Genetic algorithm software development

GA software was created and tested using Matlab R2018b (Mathworks, Inc., Matick, MA, USA). GA was created for each objective function separately. The mathematical models generated by RSM were maximized.

Bioactive content analytical methods

Total phenolic contents of verjuice vinegar samples were determined according to the spectrophotometric Folin-Ciocalteu method (Singleton and Rossi 1965). The measurement was calculated using a standard curve for gallic acid and expressed as milligrams of gallic acid equivalents (mg GAE/mL). The total flavonoid content was measured with the aluminum chloride colorimetric analysis method (Zhishen et al. 1999). Total flavonoid content was expressed as mg catechin equivalents (mg CE/mL) per liter. Determination of antioxidant capacity was completed with the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, where the DPPH radical forms based on inhibition with some modifications (Grajeda-Iglesias et al. 2016). The Cu(II) ion reducing antioxidant capacity (CUPRAC) method was used to determine the antioxidant capacity (Apak et al. 2006). Absorbance measurements were

performed by a UV–VIS spectrophotometer (SP-UV/VIS-300SRB, Spectrum Instruments, Melbourne, Australia).

Cell viability assay

For the determination of cytotoxic effects of TVV, PVV and UVV, A549 lung cancer and BEAS-2B normal lung epithelial cells were used. Cells were commercially obtained from ATCC (American Type Culture Collection) and cultivated using complete Dulbecco's modified eagle's medium (DMEM) containing 10% fetal calf serum (FCS) and 50 U/ml penicillin/streptomycin. Cells were maintained in a carbon dioxide incubator adjusted to 37 °C, 5% CO_2 and 95% humidity. For the cell viability assay, cells were cultured using 96-well culture plates at a concentration of $1x10^4$ cells per ml and leaved for overnight incubation. Subsequently, cells were treated with different concentrations (175, 87.5, 43.75, 21.87, 10.93, 5.46 µl/ml) of TVV, PVV and UVV for 48 h. After 48 h of treatment, cells were rinsed with HBSS (Hank's Balanced Salt Solution) and subjected to 1 mg/ml MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) incubation for 45–60 min at 37 °C. Following incubation, the MTT solution was discarded and plates were air-dried at room temperature. The formed formazan particles were then dissolved using 100 µl DMSO and mixed well. Plates were read using a spectrophotometer at 550 nm wavelength. All tests were studied in triplicate and cell viabilities were calculated using the "cell viability (%) = (OD sample/OD control) × 100" formula (OD: Optical Density measured at 550 nm). IC50 values for each cell were also calculated. The measured values were analyzed in GraphPad Prism6 program.

Microbiological quality

Glucose Yeast Extract Calcium Carbonate Agar (GYC, 10% glucose, 1% yeast extract, 2% calcium carbonate, 1.5% agar, pH 6.8, HiMedia, India) was seeded in vinegar samples according to the spreading method and petri dishes were incubated for 5–10 days at 30 °C (De Vero et al. 2006). For the lactic acid bacterial count, dilutions of Man Rogosa and Sharp Agar (MRS, pH 6.2, Merck, Germany) were performed according to the poured plate method and incubated at 30 °C for 3–5 days. Potato Dextrose Agar (PDA, pH 5.6, Merck, Germany) acidified with 10% tartaric acid (Merck, Germany) was seeded from the appropriate dilutions for counting of mold and yeast according to the pouring plate method and the oils were dried at 25 °C. Total Enterobacteriaceae was determined in VRBG (Violet Red Bile Glucose Agar-Merck, Germany) incubated at 37 °C for 24 h. The pink-red ring and red precipitation colonies were evaluated (Harrigan 1998). Results are given as log colony forming units (CFU) per milliliter of verjuice vinegar.

Sensory evaluation

As a result of the optimization, sensory evaluation of TVV, PVV and UVV samples were performed. Basically, it was used by making some modifications in the dictionary in a study previously developed for vinegars (Callejón et al. 2008). Previously trained panelists were preferred for sensory evaluation. The acceptance test was performed for pungent sensation, richness in aroma, general impression, taste, aromatic intensity and ethyl acetate odor, using a 9-point structured hedonic scale ranging from (1) dislike extremely to (9) like extremely. A total of 30 (18 females, 12 male) panelists evaluated the juices. Scale scores were excellent, 9; very good 8; good, 7; acceptable, 6; and poor (first odorless, tasteless development) < 6. Lower points were accepted as 6. Prior to sensory evaluation, the juice samples were cooled, randomly coded with three-digit numbers, and the order of presentation was completely randomized for each panelist.

Statistical analysis

Verjuice vinegar production was repeated three times. The analyses were performed in triplicate and expressed as mean ± standard deviation (SD). The significant differences between mean values of verjuice vinegar samples were determined by analysis of variance (one way-ANOVA) using Tukey's HSD (Honestly Significant Difference) test at a significance level of $p < 0.05$. Statistical analysis was conducted using SPSS 22.0 software (SPSS Inc., Chicago, USA). Figures for cell viability analyses were prepared with GraphPad Prism 7.0 software (GraphPad Software Inc., San Diego, CA, USA).

Results and discussion

Evaluation of bioactive components

The polynomial mathematical equation indicating the effect of time and amplitude factors on the TPC of verjuice vinegar samples is given below.

$$\begin{aligned}\text{TPC (mg GAE/L)} = {} & 392.0 + 9.44X_1 + 0.915X_2 \\ & - 0.1792X_1^2 - 0.00118X_2^2 \\ & - 0.0899X_1 \times X_2 \end{aligned} \quad (4)$$

The results of TPC (mg GAE/L) values for the samples of verjuice vinegar samples treated at different levels are shown in Table 1.

There is a good quadratic relationship between the ultrasound condition factors of the model used in the study and the total amount of flavonoid substance $R^2 = 0.9767$ (Table 1). The cross-interactions of X_1 factor applied to verjuice vinegar samples were significant ($p < 0.05$), whereas cross-interactions of X_2 factor were not statistically significant ($p > 0.05$). The two-way interactions of the factors applied to the vinegar were not statistically significant ($p > 0.001$). The three-dimensional variation of the amount of TPC with respect to time and amplitude is shown in Fig. 1a. When the model of TPC was examined, it was found that as time and amplitude values increased, it generally caused a linear increase in TPC amount. The lowest TPC was obtained with 450.71 GAE/L for sample 11 treated for 4 min and 50%; the highest TPC of 465.15 GAE/L was detected in sample 2 treated with 70% for 8 min (Table 1). At the end of the study, it was found that ultrasound-treated verjuice vinegar improved TPC values. RSM optimization detected 464.44 mg GAE/L at 9.4 min and 68.7 amplitude treatment (Table 1). The graph of the change in the suitability value for TPC according to the number of generations is shown in Fig. 2. As a result of GA optimization, the X_1 value for TPC was 9.2 and X_2 value was 68.8. As a result, TPC found with GA was 464.40 mg GAE/L in this case. RSM and GA results were close to each other. At the end of RSM optimization, it was found that the TPC amount increased by 3.7% compared to the TVV sample. In the pasteurization process, it was determined that the TPC amount of the TVV sample decreased by 14%.

The polynomial mathematical equation indicating the effect of time and amplitude factors on TFC value of verjuice vinegar samples is given below.

$$\begin{aligned}\text{TFC (mg CE/L)} = {} & 59.32 + 0.603X_1 + 0.1969X_2 \\ & - 0.00301X_1^2 - 0.000909X_2^2 \\ & - 0.00562X_1 \times X_2 \end{aligned} \quad (5)$$

It is seen that there is a good quadratic relationship between the ultrasound condition factors of the model used in the study and the total amount of flavonoid substance

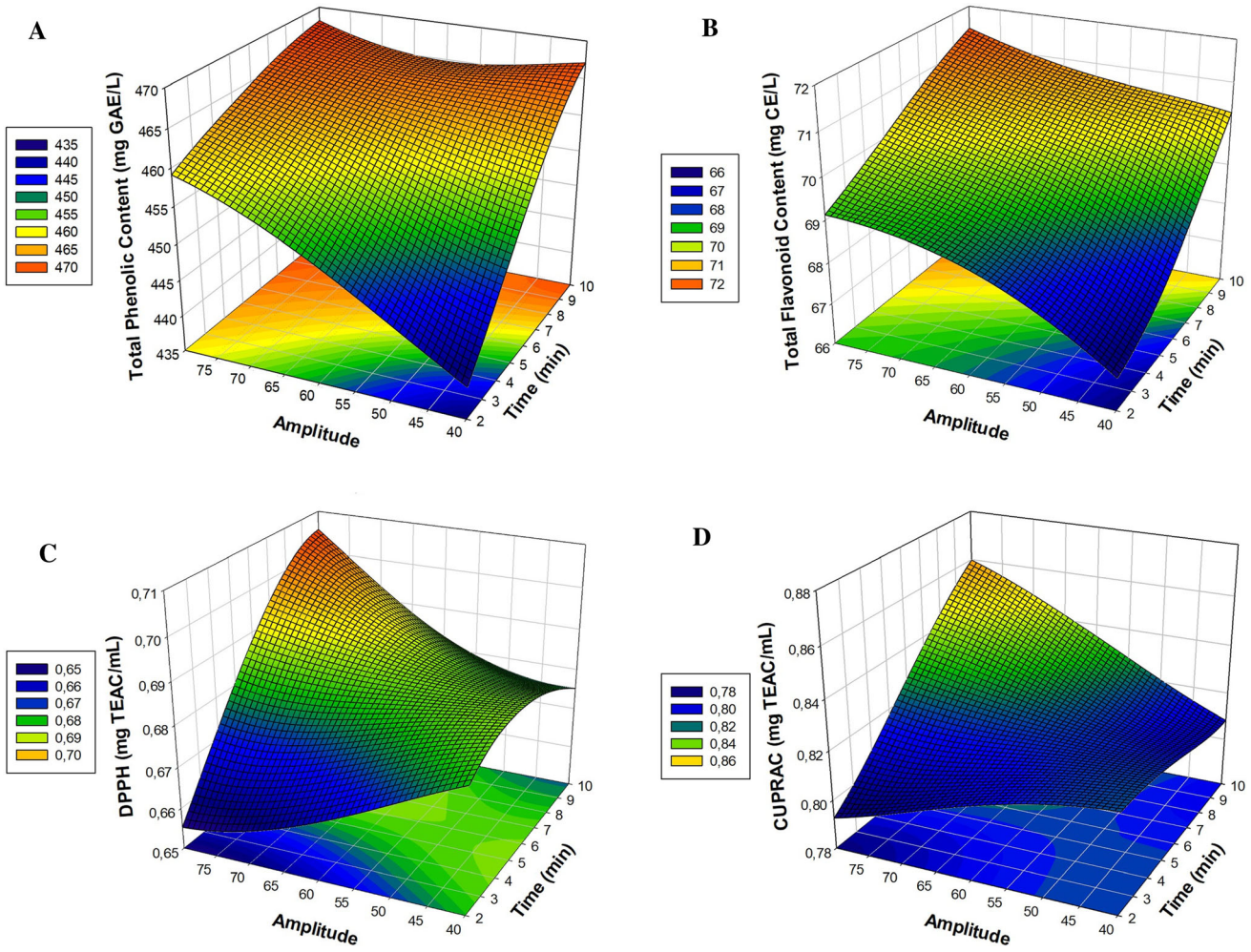

Fig. 1 Response surface plots (3D) of TPC (**a**), TFC (**b**), DPPH (**c**) and CUPRAC (**d**) analysis as a function of significant interaction factors

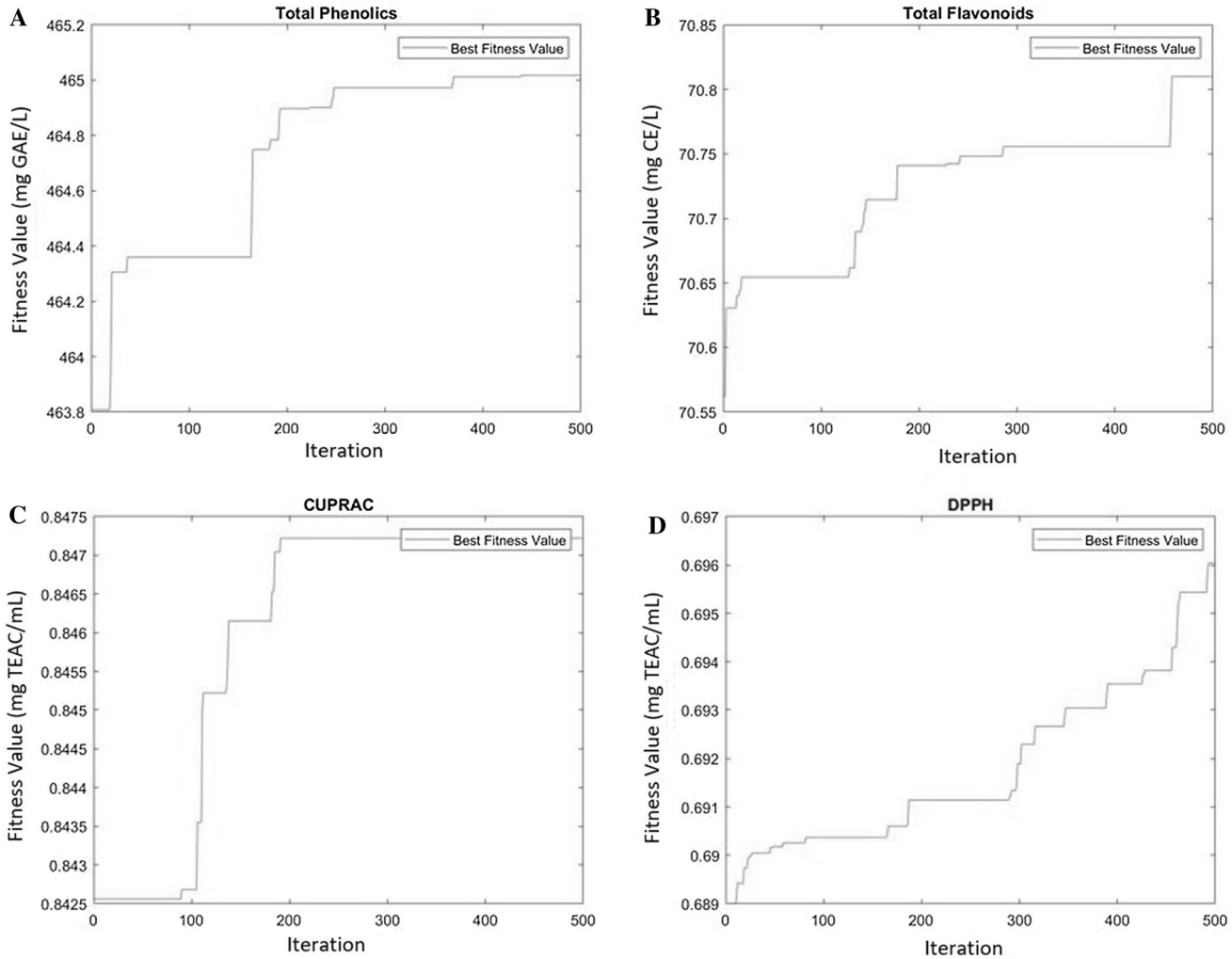

Fig. 2 Change in suitability value according to the number of generations TPC (**a**), TFC (**b**), DPPH (**c**) and CUPRAC (**d**)

$R^2 = 0.9754$ (Table 1). Linear effects of X_1 and X_2 factors on TFC values for verjuice vinegar samples were found to be statistically significant ($p < 0.001$). The three-dimensional variation in the amount of TFC relative to time and amplitude is shown in Fig. 1b. When the model for TFC was examined, it was found that as time and amplitude values increased, it generally caused a linear increase in TFC amounts. The lowest TFC of 68.04 CE/L was in sample 13 with 6 min and 40% treatment; the highest value of 70.68 CE/L was detected in sample 8 treated with 60% for 10 min (Table 1). The graph of change in the conformity value created for TFC according to the number of generations is shown in Fig. 2. As a result of GA optimization, the X_1 value for TFC was 9.4 and X_2 was 61.12. As a result, TFC value with GA was 70.66 mg CE/L in this case. At the end of the study, it was found that ultrasound treatment of verjuice vinegar improved the TFC values. RSM optimization detected 70.85 mg CE/L at 9.4 min and 68.7 amplitude treatment (Table 1). At the end of the optimization, it was determined that the amount of TFC increased by 9.1% compared to the TVV sample. With the pasteurization process, it was determined that the TFC amount in the TVV sample decreased by 7.6%. Thanks to the ultrasound assisted extraction treatment of olive kernel, improvements in phenolic substances were detected (Roselló-Soto et al. 2015a). The increase in TPC and TFC of apple juice to which ultrasound treatments were applied compared to the control sample was reported by researchers to be due to the addition of hydroxyl radicals to the aromatic ring of phenolic compounds and breakage of cell walls with the effect of cavitation (Aadil et al. 2013). Similar pulses were detected in ultrasound treatments applied to Kasturi lime juice, and they may be associated with the deterioration of cell walls (Bhat et al. 2011). In addition, ultrasound treatments can contribute to removal of the active oxygen stuck in the juice and development of phenolic compounds (Masuzawa et al. 2000). In our study, as stated in the previous reports, the cavitation caused by

the micro shock waves created by ultrasound treatments caused more disruption of the cells and caused an increase in TPC and TFC.

The polynomial mathematical equation indicating the effect of time and amplitude factors on the DPPH (mg TEAC/mL) of verjuice vinegar samples is given below.

$$\begin{aligned}\text{DPPH (mg TEAC/mL)} = {} & 07515 - 0.00515X_1 \\ & - 0.002068X_2 - 0.000669X_1^2 \\ & - 0.000005X_2^2 - 0.000262X_1 \\ & \times X_2\end{aligned} \quad (6)$$

There is a good quadratic relationship between ultrasound condition factors and DPPH amount of the model used in the study $R^2 = 0.9909$ (Table 1). The linear effects of X_1 and X_2 factors on DPPH values of verjuice vinegar samples were found to be statistically significant ($p < 0.001$). The three-dimensional variation of the DPPH amount with respect to time and amplitude is shown in Fig. 1c. When the model for DPPH was examined, it was found that as time and amplitude values increased, it caused a general linear increase in DPPH amounts. The lowest DPPH was 0.664 TEAC/mL in sample 4 treated for 2 min and 60%; maximum 0.694 TEAC/mL was detected in sample 2 treated with 70% for 8 min (Table 1). The graph of the change of the suitability value for the DPPH values according to the number of generations is shown in Fig. 2. As a result of GA optimization, the X_1 value was 9.3 and X_2 value was 70 for DPPH. As a result, DPPH found with GA was 0.696 mg TEAC/mL. RSM and GA results were close to each other.

The polynomial mathematical equation indicating the effect of time and amplitude factors on CUPRAC (mg TEAC/mL) values of verjuice vinegar samples is given below.

$$\begin{aligned}\text{CUPRAC (mg TEAC/mL)} = {} & 09417 - 0.021865X_1 \\ & - 0.002538X_2 \\ & - 0.000212X_1^2 \\ & - 0.000005X_2^2 \\ & - 0.000376X_1 \times X_2\end{aligned} \quad (7)$$

It is seen that there is a good quadratic relationship between ultrasound condition factors and CUPRAC amount of the model used in the study $R^2 = 0.9937$ (Table 1). Linear effects of X_1 and X_2 factors on CUPRAC values of verjuice vinegar samples were found to be statistically significant ($p < 0.001$). The three-dimensional variation in the amount of CUPRAC with respect to time and amplitude is shown in Fig. 1d. When the model for CUPRAC was examined, it was found that as time and amplitude values increased, it generally caused a linear increase in CUPRAC amounts. The lowest CUPRAC was 0.808 TEAC/mL in sample 9 treated for 4 min and 70%; highest 0.837 TEAC/mL was detected in sample 2 treated with 70% for 8 min (Table 1). At the end of the study, it was found that ultrasound treatment of verjuice vinegar improved CUPRAC values. RSM optimization found 0.846 TEAC/mL after 9.4 min and 68.7 amplitude treatment (Table 1). The graph of change for the conformity value created for CUPRAC values according to the number of generations is shown in Fig. 2. As a result of GA optimization, the X_1 value was 9.4 for CUPRAC and X_2 value was 70 for CUPRAC. As a result, CUPRAC with GA was 0.849 mg TEAC/mL in this case. RSM and GA results were close to each other. At the end of the optimization, it was determined that the amount of CUPRAC increased by 6% compared to the TVV sample. With the pasteurization process, the amount of CUPRAC in the TVV sample decreased by 4.7%. These results indicate that ultrasound treatment increases the extractability of antioxidant compounds. Purple cactus juice and Kasturi lime juice with different amplitude time treatments applied had an increase in the total antioxidant amount release, as in our study (Bhat et al. 2011; Zafra-Rojas et al. 2013). Also, the possible cause of the observed increase can be explained by the addition of hydroxyl radicals (OH) produced by sound waves to the aromatic ring of phenolic compounds. The addition of a second hydroxyl group was reported to increase the total antioxidant activity of ortho- or para-molecules (Masuzawa et al. 2000).

Anticarcinogenic activity

To reveal the cytotoxic effects of vinegar samples obtained by different techniques, we used the MTT cell viability assay. Verjuice vinegar obtained by the traditional method was used as a control. Accordingly, the effects of vinegar improved by pasteurization and ultrasound techniques on cell viability were evaluated. In A549 cancer cells, while vinegar samples obtained by TVV and UVV were effective at 87.5 and 175 µl/ml concentrations, samples obtained by PVV were found to be effective only at the highest concentration (Fig. 3a–c). When A549 cells were treated with TVV, PVV and UVV, IC50 values after 48 h were found to be 63.99, 120 and 117 µl/ml, respectively. Analysis of BEAS-2B normal lung cells revealed that TVV and PVV-treated vinegar samples had similar results as A549 cells, but UVV-treated vinegar samples only affected cells at the highest concentration (175 µl/ml) (Fig. 3d–f). When the BEAS-2B cells were treated with TVV, PVV and UVV, IC50 values after 48 h were calculated to be 62.46, 98.49 and 99.39 µl/ml, respectively.

In the present study we also evaluated anti-proliferative activities of PVV and UVV on lung cancer cells as well as normal lung epithelial cells. Mounting evidence indicates

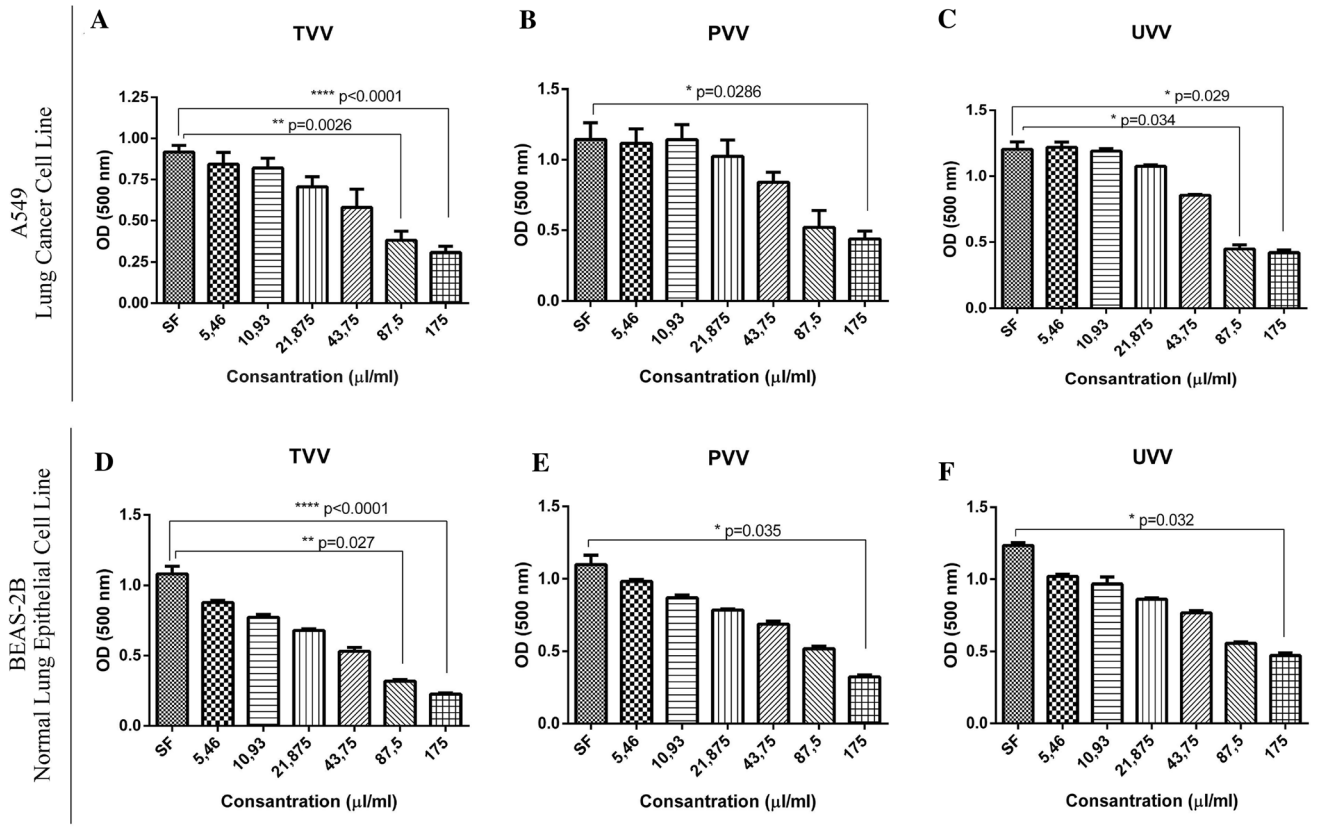

Fig. 3 The effect of TVV, PVV and UVV samples on cell viability of lung cancer and normal lung epithelial cells. **a** The effect of TVV on cell viability in A549 cells, **b** Effect of PVV on cell viability in A549 cells, **c** Effect of UVV on cell viability in A549 cells, **d** Effect of TVV on cell viability in BEAS-2B cells, **e** Effect of PVV on cell viability in BEAS-2B cells, **f** Effect of UVV on cell viability in BEAS-2B cells

that vinegar obtained from different fruit species might have significant therapeutic activities against various cancers. Particularly, it was shown that the vinegar obtained from coconut water shows dose-dependent anti-cancer effect and stimulates cancer-related inflammation and antitumor immunity in breast cancer cells (Mohamad et al. 2019). Likewise, Japanese black vinegar, called "ızumi", was reported to suppress the proliferation of human squamous cell carcinoma cells via necroptosis (Baba et al. 2013) https://www.tandfonline.com/doi/full/10.1080/01635581.2013.815234?src=recsys. In contrast to these findings, Shanxi aged vinegar (SAV) was reported to protect against ethanol-induced killing of normal liver cell line L-O2, indicating significant positive activity of this vinegar on cellular proliferation (Xia et al. 2018). Consistently, persimmon vinegar polyphenols (PVP) were demonstrated to protect against H2O2-induced cytotoxicity in HepG2 cell lines (Xia et al. 2018, 2019). In addition, persimmon vinegar polyphenols (PVP) were demonstrated to protect against H2O2-induced cytotoxicity in Hep G2 cell lines (Zou et al. 2018). Unlike protective effects, Shanxi aged vinegar extract was also shown to have no marked effect on cell viability of HepG2 hepatocellular carcinoma cells (Xia et al. 2019). In consistent with these findings, in our study, vinegar obtained by pasteurization and ultrasound techniques slightly, but not significantly, inhibited cell proliferation in contrast to TVV. We also found that the vinegar obtained by pasteurization and ultrasound techniques had no lung cancer specific activity. Also, these results suggest that pasteurization and ultrasound techniques seem to alleviate the destructive effects of vinegar samples. Consequently, here we demonstrated that vinegar samples obtained by pasteurization and ultrasound techniques have no marked effect on cell proliferation of lung cancer cells as well as normal lung epithelial cells. Nevertheless, future detailed studies are needed to fully elucidate the effects of these vinegar samples on cell lines and reveal more about the biological roles of these samples.

Microbiological quality and evaluation of sensory properties

Microbiological properties of vinegar samples are given in Table 2. While the number of acetic acid bacteria was 2.75 log CFU/mL in the TVV sample, it was 1.74 log CFU/mL in the PVV sample ($p < 0.05$). The number of lactic acid

Table 2 Effects of thermal treatment and ultrasound on microbial inactivation analysis of verjuice vinegar

Samples	Microbiology			
	Total acetic acid bacteria count (log CFU/mL)	Total Enterobacteriaceae count (log CFU/mL)	Total lactic acid bacteria count (log CFU/mL)	Yeast and mold count (log CFU/mL)
PVV	1.74 ± 0.10^{a}	ND	1.79 ± 0.05^{a}	1.18 ± 0.12^{a}
TVV	2.75 ± 0.10^{b}	ND	2.89 ± 0.25^{b}	1.28 ± 0.06^{a}
UVV	1.90 ± 0.13^{a}	ND	1.97 ± 0.14^{a}	<1

ND not detected, *CFU* colony-forming unit, *PVV* pasteurized verjuice vinegar; *TVV* traditional verjuice vinegar; *UVV* ultrasound-treated verjuice vinegar. Values followed by different letters within the same column are significantly different ($p < 0.05$) (n = 3 ± SD)

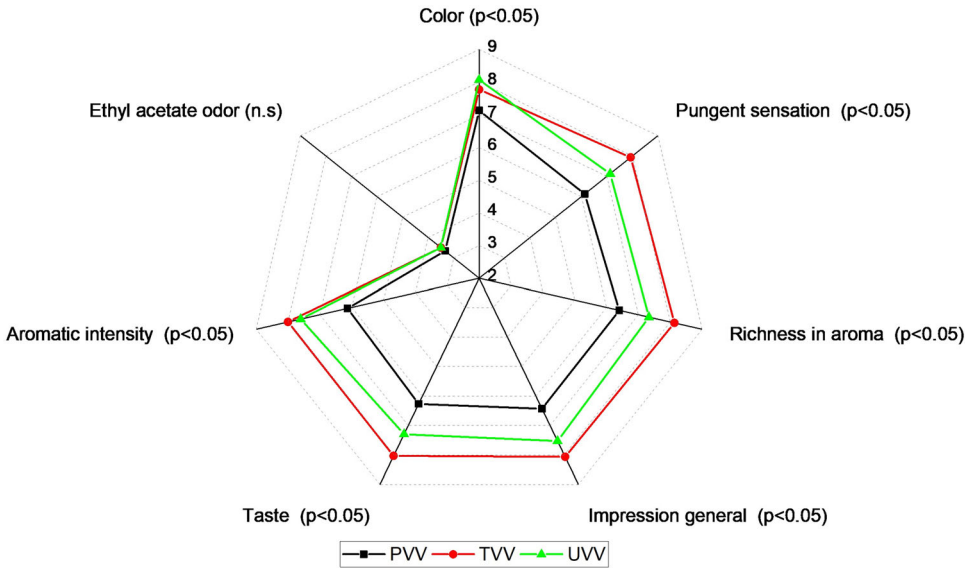

Fig. 4 Results of sensory analysis chart for treated verjuice vinegar. There were statistically significant differences between samples ($p < 0.05$). n.s: no statistical difference; *PVV* Pasteurized verjuice vinegar; *TVV* Traditional verjuice vinegar; *UVV* Ultrasound-treated verjuice vinegar

bacteria (1.97 log CFU/mL) in the UVV sample was higher than the PVV sample (1.79 log CFU/mL) ($p > 0.05$). Total Enterobacteriaceae was not detected in verjuice vinegar samples. According to our literature research, no study was found to examine the microbiological characteristics of verjuice vinegar. However, in a study examining the microbiological properties of different varieties of vinegar produced traditionally and commercially, it was reported that the number of acetic acid bacteria, lactic acid bacteria and mold-yeasts were higher in the traditionally produced vinegars compared to industrial vinegars (Ozturk et al. 2015). When the results are examined, ultrasound treatment may be an alternative to thermal pasteurization with positive properties on other quality parameters.

For sensory analysis, ultrasound-treated verjuice vinegar UVV (9.4 min and 68.7 amplitude), TVV and PVV samples were compared according to RSM results. The sensory analysis results are shown in Fig. 4. In the evaluation made by the panelists, the UVV sample was more favored than the other samples with a mean of 8.07 and statistically significant differences were found ($p < 0.05$). The PVV sample was less favorable than the other samples with a mean of 6.13 in the evaluation of pungent sensation of the samples. TVV sample was more favorable for richness in aroma sensory evaluation with average of 8.13 than the other samples and statistically significant differences were found ($p < 0.05$). General impression evaluation showed statistically significant differences between samples and the most popular TVV (8.07) was preferred by the panelists. Taste evaluation found PPV was the least preferred, although the liking of the samples was above average. Among the samples, TVV was determined the most favorable in the evaluation of aromatic intensity. Ethyl acetate odor was not statistically different between all samples ($p > 0.05$). The researchers found that ultrasound studies about carrot-grapes (Nadeem et al. 2018), orange juice (Samani et al. 2015) and cranberry juice (Jambrak et al. 2017) found that sensory properties were generally acceptable compared to other treatments. It was stated that the cavitation caused by ultrasound application may contribute to the improvement of sensory parameters due to oxygen removal from fruit juice (Samani et al. 2015). Our

study was in parallel with the literature and sensory properties were not significantly affected by ultrasound treatment and the level of acceptability was high.

Conclusion

In this study, optimization of process conditions for ultrasound treatment in terms of bioactive components of verjuice vinegar was successfully performed with RSM and GA. At the same time, the optimized ultrasound-treated verjuice vinegar (9.4 min and 68.7 amplitude), conventional verjuice vinegar and pasteurized verjuice vinegar were evaluated for the anticancerogenic, microbiological quality and sensory properties of the samples. The results of the analysis showed that the ultrasound-treated verjuice vinegar was enriched with bioactive components compared to other samples. UVV was found to be microbially safe and at the same time sensory properties were not significantly affected by ultrasound treatment and the level of acceptability was high. Verjuice vinegar was found to have anticarcinogenic effects and there was no significant difference between the samples. However, further research into the mechanisms of action of the anticarcinogenic activity of verjuice vinegar will play an important role in health. Further research involving experimental animal models and human clinical trials should support the results of this study.

Acknowledgements Bioactive component analyzes, microbiology, sensory analyzes and RSM optimization were performed by Seydi Yıkmış and also made statistical analyzes of these sections. GA optimization was performed by Mehmet Ali Şimşek. Anticarcinogenic activity analysis and statistics were performed by Esra Bozgeyik.

Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

References

Aadil RM, Zeng X-A, Han Z, Sun D-W (2013) Effects of ultrasound treatments on quality of grapefruit juice. Food Chem 141:3201–3206. https://doi.org/10.1016/J.FOODCHEM.2013.06.008

Abid M, Jabbar S, Hu B et al (2014) Thermosonication as a potential quality enhancement technique of apple juice. Ultrason Sonochem 21:984–990. https://doi.org/10.1016/J.ULTSONCH.2013.12.003

Apak R, Güçlü K, Özyürek M et al (2006) The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. Int J Food Sci Nutr 57:292–304. https://doi.org/10.1080/09637480600798132

Baba N, Higashi Y, Kanekura T (2013) Japanese black vinegar "izumi" inhibits the proliferation of human squamous cell carcinoma cells via necroptosis. Nutr Cancer 65:1093–1097. https://doi.org/10.1080/01635581.2013.815234

Bhat R, Kamaruddin NSBC, Min-Tze L, Karim AA (2011) Sonication improves kasturi lime (Citrus microcarpa) juice quality. Ultrason Sonochem 18:1295–1300. https://doi.org/10.1016/J.ULTSONCH.2011.04.002

Callejón RM, Morales ML, Silva Ferreira AC, Troncoso AM (2008) Defining the typical aroma of Sherry vinegar: sensory and chemical approach. J Agric Food Chem 56:8086–8095. https://doi.org/10.1021/jf800903n

Chemat F, Khan MK (2011) Applications of ultrasound in food technology: processing, preservation and extraction. Ultrason Sonochem 18:813–835. https://doi.org/10.1016/j.ultsonch.2010.11.023

Chen H, Chen T, Giudici P, Chen F (2016) Vinegar functions on health: constituents, sources, and formation mechanisms. Compr Rev Food Sci Food Saf 15:1124–1138. https://doi.org/10.1111/1541-4337.12228

De Vero L, Gala E, Gullo M et al (2006) Application of denaturing gradient gel electrophoresis (DGGE) analysis to evaluate acetic acid bacteria in traditional balsamic vinegar. Food Microbiol 23:809–813. https://doi.org/10.1016/j.fm.2006.01.006

Deb K (2012) Optimization for engineering design: algorithms and examples, Second Ed. Prentice Hall, Upper Saddle River

Gao S, Lewis GD, Ashokkumar M, Hemar Y (2014) Inactivation of microorganisms by low-frequency high-power ultrasound: 1. Effect of growth phase and capsule properties of the bacteria. Ultrason Sonochem 21:446–453. https://doi.org/10.1016/j.ultsonch.2013.06.006

Grajeda-Iglesias C, Salas E, Barouh N et al (2016) Antioxidant activity of protocatechuates evaluated by DPPH, ORAC, and CAT methods. Food Chem 194:749–757. https://doi.org/10.1016/j.foodchem.2015.07.119

Gullo M, Verzelloni E, Canonico M (2014) Aerobic submerged fermentation by acetic acid bacteria for vinegar production: process and biotechnological aspects. Process Biochem 49:1571–1579

Harrigan WF (1998) Laboratory methods in food microbiology, 3rd edn. Academic Press, New York

Jambrak AR, Šimunek M, Petrović M et al (2017) Aromatic profile and sensory characterisation of ultrasound treated cranberry juice and nectar. Ultrason Sonochem 38:783–793. https://doi.org/10.1016/J.ULTSONCH.2016.11.027

Karabiyikli S, Sengun IY (2017) Beneficial effects of acetic acid bacteria and their food products. Acetic Acid Bacteria: Fundamentals and Food Applications. CRC Press, Boca Raton, pp 221–242

Mason TJ, Paniwnyk L, Chemat F (2003) Ultrasound as a preservation technology. In: Zeuthen P, Bøgh-Sørensen L (eds) Food preservation techniques. Woodhead Publishers, Sawston, pp 303–337

Masuzawa N, Ohdaira E, Ide M (2000) Effects of ultrasonic irradiation on phenolic compounds in wine. Jpn J Appl Phys 39:2978–2979. https://doi.org/10.1143/JJAP.39.2978

Mohamad NE, Yeap SK, Abu N et al (2019) In vitro and in vivo antitumour effects of coconut water vinegar on 4T1 breast cancer cells. Food Nutr Res. https://doi.org/10.29219/fnr.v63.1616

Nadeem M, Ubaid N, Qureshi TM et al (2018) Effect of ultrasound and chemical treatment on total phenol, flavonoids and antioxidant properties on carrot-grape juice blend during storage. Ultrason Sonochem 45:1–6. https://doi.org/10.1016/J.ULTSONCH.2018.02.034

Ozturk I, Caliskan O, Tornuk F et al (2015) Antioxidant, antimicrobial, mineral, volatile, physicochemical and microbiological characteristics of traditional home-made Turkish vinegars. LWT Food Sci Technol 63:144–151. https://doi.org/10.1016/j.lwt.2015.03.003

Pazuch CM, Siepmann FB, Canan C, Colla E (2015) Vinegar: functional aspects. Científica 43:302. https://doi.org/10.15361/1984-5529.2015v43n4p302-308

Rawson A, Patras A, Tiwari BK et al (2011) Effect of thermal and non thermal processing technologies on the bioactive content of exotic fruits and their products: review of recent advances. Food Res Int 44:1875–1887. https://doi.org/10.1016/J.FOODRES.2011.02.053

Rojas ML, Miano AC, Augusto PED (2017) Ultrasound processing of fruit and vegetable juices. Ultrasound Adv Food Process Preserv. https://doi.org/10.1016/B978-0-12-804581-7.00007-5

Roselló-Soto E, Barba FJ, Parniakov O et al (2015a) High voltage electrical discharges, pulsed electric field, and ultrasound assisted extraction of protein and phenolic compounds from olive kernel. Food Bioprocess Technol 8:885–894. https://doi.org/10.1007/s11947-014-1456-x

Roselló-Soto E, Galanakis CM, Brnčić M et al (2015b) Clean recovery of antioxidant compounds from plant foods, by-products and algae assisted by ultrasounds processing. Modeling approaches to optimize processing conditions. Trends Food Sci Technol 42:134–149

Salleh-Mack SZ, Roberts JS (2007) Ultrasound pasteurization: the effects of temperature, soluble solids, organic acids and pH on the inactivation of Escherichia coli ATCC 25922. Ultrason Sonochem 14:323–329. https://doi.org/10.1016/J.ULTSONCH.2006.07.004

Samani BH, Khoshtaghaza MH, Lorigooini Z et al (2015) Analysis of the combinative effect of ultrasound and microwave power on Saccharomyces cerevisiae in orange juice processing. Innov Food Sci Emerg Technol 32:110–115. https://doi.org/10.1016/J.IFSET.2015.09.015

Singleton V, Rossi A (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. Am J Enol Vitic 16:144–158

Siwach R, KumarKumar M (2012) Comparative study of thermosonication and thermal treatments on pectin methyl esterase inactivation in mosambi juice. J Dairying Foods Home Sci 31:290–296

Xia T, Zhang J, Yao J et al (2018) Shanxi aged vinegar prevents alcoholic liver injury by inhibiting CYP2E1 and NADPH oxidase activities. J Funct Foods 47:575–584. https://doi.org/10.1016/j.jff.2018.06.018

Xia T, Zhang B, Duan W et al (2019) Hepatoprotective efficacy of Shanxi aged vinegar extract against oxidative damage in vitro and in vivo. J Funct Foods. https://doi.org/10.1016/j.jff.2019.103448

Zafra-Rojas QY, Cruz-Cansino N, Ramírez-Moreno E et al (2013) Effects of ultrasound treatment in purple cactus pear (Opuntia ficus-indica) juice. Ultrason Sonochem 20:1283–1288. https://doi.org/10.1016/J.ULTSONCH.2013.01.021

Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 64:555–559. https://doi.org/10.1016/S0308-8146(98)00102-2

Zoran H, Anet Režek J, Vesna L, Selma Mededovic T (2012) The effect of high intensity ultrasound treatment on the amount of *Staphylococcus aureus* and *Escherichia coli* in Milk. Food Technol Biotechnol 50:46–52

Zou B, Xiao G, Xu Y et al (2018) Persimmon vinegar polyphenols protect against hydrogen peroxide-induced cellular oxidative stress via Nrf2 signalling pathway. Food Chem 255:23–30. https://doi.org/10.1016/j.foodchem.2018.02.028