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Received: 20 September 2018 | Revised: 3 April 2019 | Accepted: 4 May 2019

DOI: 10.1111/jfpe.13131

ORIGINAL ARTICLE

Journal of Food Process Engineering

WILEY

Pasteurization of verjuice by UV-C irradiation and mild heat treatment

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Funding information
IZTECH Biotechology and Bioengineering Research and Application Center; Izmir Institute of Technology, Grant/Award Numbers: 2015-IYTE-08, IZTECH-BAP

Abstract

Verjuice is a highly acidic juice and more prone to yeast spoilage. In this study, the efficacy of individual and combination processes of UV-C irradiation (UV) and mild heat treatment (MH) for pasteurization of verjuice were assessed. *Saccharomyces cerevisiae* (NRRL Y-139) was selected as the target microorganism and kinetic parameters for MH, UV, and combined UV + MH inactivation treatments were determined. The UV treatment alone at a UV dose of 0.57 J/cm^2 (energy of 2.30 J/mL), provided only 0.54 ± 0.02 log CFU/mL reduction of *S. cerevisiae*. In contrast, the combined treatment (UV + MH2) substantially reduced the number of *S. cerevisiae* in verjuice, 5.16 ± 0.24 log CFU/mL reduction was achieved at 0.25 J/cm^2 UV dose (energy of 1.01 J/mL) and 51.25 ± 1.47°C. The percentage of synergism for the UV + MH inactivation of *S. cerevisiae* in verjuice was maximized at 51.25°C (50.79% of synergistic effect). Inactivation kinetics of *S. cerevisiae* was best described by Weibull model with the smallest RMSE and AIC values. *D* value was decreased from 13.66 to 1.94 min when UV was combined with mild heating. The results showed that UV-C light assisted by mild heat treatment can be a potential alternative to thermal pasteurization of verjuice.

Practical applications

Fruit juices are prone to spoilage by yeasts, molds, and some acid-tolerant bacteria. *Saccharomyces cerevisiae* is a heat resistant spoilage microorganism and found in some spoiled juices. Thermal pasteurization is widely used for the preservation of fruit juices but results in losses of essential nutrients and changes in physicochemical and organoleptic properties. This study illustrated that the combined UV-C light assisted by mild heat treatment can deliver the required microbial reduction in verjuice. The synergistic effect of two processing methods is suggested for controlling the growth of spoilage microflora of fruit juices.

1 | INTRODUCTION

Verjuice (unripe juice) is a grape juice obtained from unripe green grapes. It has a specific aroma and sour taste (Hayoglu, Kola, Kaya, Ozer, & Turkoglu, 2009). Verjuice is a savory alternative to vinegar and lemon juice because it has the same acid-base equilibrium (Oncul & Karabiyikli, 2015). It is used generally in traditional meals, vegetable salads, and snacks to give flavor, and mixed as an ingredient in several alcoholic beverages and sauces (Karapinar & Sengun, 2007). Verjuice has been known as an antimicrobial and antioxidant as well as a traditional medicine (Aminian, Aminian, & Hoseinali, 2006; Karapinar & Sengun, 2007; Setorki, Asgary, Eidi, & Rohani, 2010). It is

Zehra Kaya and Sevcan Unluturk contributed equally to this work.

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usually made at home without using any technology. Thus, it is prone to spoilage by yeasts and molds grown naturally in grapes. Therefore, it has a short shelf life (Hayoglu et al., 2009).

Thermal pasteurization, which is the most common preservation method applied below 100°C, provides long and stable shelf life for fruit juices by reducing pathogen and spoilage microorganisms (Rivas, Rodrigo, Martinez, Barbosa-Canovas, & Rodrigo, 2006). However, it causes several sensorial and nutritional quality problems such as loss of flavor and taste, degradation of nutrients, and undesirable browning reactions (Garde-Cerdan, Arias-Gil, Marselles-Fontanet, Ancin-Azpilicueta, & Martin-Belloso, 2007; Walkling-Ribeiro et al., 2008). UV-C irradiation has been used efficiently to inactivate microorganism in liquid foods. This technology has been approved by the Food and Drug Administration for fruit juice pasteurization (Food and Drug Administration [FDA], 2000). UV-C light (especially at 253.7 nm) has a germicidal effect on microorganisms and prevents transcription and reproduction of the cells by forming dimers on thymine structure in their DNA (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000). UV-C irradiation is successfully used for water and air disinfection, and surface decontamination in meat, poultry, and seafood products (Koutchma, 2008; Shah, Shamsudin, Rahman, & Adzahan, 2016). UV treatment systems are cost efficient, easy to install, and compatible with other devices such as filters (Bintsis et al., 2000; Johnson, Kumar, Ponmurugan, & Gananamangai, 2010; Pereira & Vicente, 2010). Recent studies reported that UV-C irradiation can be used as an alternative to thermal pasteurization without any change in the physicochemical, nutritional, and sensorial quality of the juices (Chia, Rosnah, Noranizan, & Ramli, 2012; Kaya, Yildiz, & Unluturk, 2015; Muller, Noack, Greiner, Stahl, & Posten, 2014; Santhirasegaram, Razali, George, & Somasundram, 2015). However, the application of UV-C irradiation is restricted for certain fruit juices due to the presence of high amount of color compounds and soluble and/or suspended particles, which reduce the penetration ability of UV light (Koutchma, Keller, Chirtel, & Parisi, 2004).

Hurdle technologies, which involve the application of a combination of emerging preservation treatments or conventional treatments at mild conditions, have become popular in recent years. The main target of hurdle approach is to obtain microbially safe products using mild effect of temperature, pH, water activity, antimicrobials, and other nonthermal processes (Leistner & Gorris, 1995; Ross, Griffiths, Mittal, & Deeth, 2003; Shah et al., 2016). Many studies have been conducted with fruit juices using a combination of nonthermal technologies such as pulse light—ultrasound (Ferrario, Alzamora, & Guerrero, 2015); ultrasound—high hydrostatic pressure (Abid et al., 2014); UV-C irradiation—pulse electric field (PEF; Noci et al., 2008); and PEF—high intensity light pulses (Caminiti et al., 2011). It has also been reported that the inactivation efficacy of UV-C irradiation in fruit juices can be increased by combining UV-C irradiation with mild heating at different temperatures (Carrillo, Ferrario, & Guerrero, 2017; Gayan, Manas, Alvarez, & Condon, 2013; Gayan, Serrano, Alvarez, & Condon, 2016; Gayan, Serrano, Monfort, Alvarez, & Condon, 2012; Gayan, Torres, Alvarez, & Condon, 2014; Gouma, Gayan, Raso, Condon, & Alvarez, 2015).

The main objective of this research was to assess the potential of individual UV-C irradiation (UV) and the combination processes of UV-C irradiation and mild heat treatment (MH) for pasteurization of verjuice by investigating their impact on the inactivation kinetics of *Saccharomyces cerevisiae*, which was selected as the target spoilage microorganism in verjuice.

2 | MATERIALS AND METHODS

2.1 | Verjuice

Yediveren variety of unripe grapes (*Vitis vinifera L.*) was purchased from vineyards in Urla region of Izmir, Turkey. The ripening degree of the grapes was determined from the maturity index (Equation 1; Palomo, Diaz-Maroto, Vinas, Soriano-Perez, & Perez-Coello, 2007).

$$\text{Maturity index} = \frac{\text{Soluble solid content } (^\circ\text{brix})}{\text{Acidity } (\text{g/L})} \quad (1)$$

Total soluble solid content (°Bx) of the unripe grapes was measured by means of a hand-held refractometer (Pocket refractometer PAL-1, Atago, Co, Ltd., Tokyo Tech., Japan) at 20°C. The titratable acidity (TA) was determined according to the method of AOAC (1990) and expressed as the weight of tartaric acid in 100 mL juice (wt/vol). A pH meter (Inolab 7310, WTW series, Germany) was used to determine pH values of samples at 20°C.

Unripe grapes were first separated from the stems. The berries were then washed with 5% grape vinegar solution (Kukre Gida A.S., Turkey; containing 0.25% wt/vol acetic acid) and deionized water (2 L/kg grapes), respectively, to remove any foreign particles such as leaves and soil from the surface of the grapes and to reduce initial microbial load of the grapes. After removing of excessive water on the surface of the grapes (surface drying for 50°C/15 min), they were stored at the freezer (−20°C) until used.

Verjuice was obtained after defrosting, extraction, and detartarization steps. Grape berries were first defrosted. Then a household tabletop fruit juice extractor (Arçelik, Robolio, Istanbul) was used to extract the juice. After juice extraction, the verjuice was stored in the refrigerator (4°C) for 24 hr to precipitate the tartrate particles (10–15% vol/vol). In the last step, the juice was filtered through the sterile double layer cheese cloths to obtain clear verjuice.

2.1.1 | Optical properties and color

Optical properties were also measured before treatments. The absorption coefficient of the verjuice was estimated from the measurement of absorbance values using a Carry 100 U Visible Spectrophotometer (Varian, Inc., CA) set at a wavelength of 254 nm. Turbidimeter (Model 2100AN IS, HACH Company) was used for measurement of cloudiness of the juice and expressed as Nephelometric Turbidity Unit (NTU). CIE color parameters expressed as L^* (brightness-darkness), a^* (redness-greenness), b (yellowness-blueness) were determined using Konica Minolta CR 400 Chromometer (Konica, Inc., Japan).

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2.2 | Target microorganism

Verjuice is an acidic product (pH 2.6–2.7) and the main spoilage microbiota is dominated by yeasts. Thus, *S. cerevisiae* was selected as the target microorganism for verjuice processing. Gabriel (2012) suggested applying 5D concept for the spoilage microorganism that shows the greatest growth rate in storage. Therefore, a 5-log CFU/mL reduction of *S. cerevisiae* in verjuice was aimed to mimic the worst scenario condition in verjuice.

The yeast culture was maintained frozen at −80°C in cryovials containing 25% glycerol. Initial inoculum was prepared by transferring 100 μL of cells from the cryovial to 100 mL yeast-extract-peptone-dextrose (YPD) broth (pH 6.55). Incubation was performed at 30°C and 200 rpm for 24 hr to obtain a stock culture. Next, *S. cerevisiae* cells were adapted to high acidic conditions by gradually reducing pH of the YPD broth with the addition of 10% tartaric acid (Merck, Darmstadt, Germany). For this purpose, 100 μL of *S. cerevisiae* from stock culture was first inoculated into 100 mL YPD broth (pH 3.5) and incubated in an orbital shaker (Thermo Electron Corp., OH) at 30°C and 200 rpm for 24 hr. Then, 1 mL of yeast cells was transferred into YPD broth adjusted to pH of 2.7 and incubated in the shaker for 48 hr at the same conditions (30°C and 200 rpm). At the end of this acid adaptation and enrichment stage, cells were transferred on PDA slants and stored in a refrigerator at 4°C until used.

Background microbiota of the verjuice, that is, total mesophilic aerobic, yeasts and molds, and total coliforms were counted by spread plating on the plate count agar (PCA, Merck, Darmstadt, Germany), the acidified PDA (pH 3.5) and violet red bile agar (VRBA, Merck, Darmstadt, Germany) plates, respectively. PCA, PDA, and VRBA plates were incubated at 30°C for 48 hr, at 25°C for 2–5 days, and at 37°C for 24 hr, respectively. Enumeration of *S. cerevisiae* was done on acidified PDA (pH 3.5) by incubating at 30°C for 2–5 days. Results were expressed as CFU/mL.

2.3 | UV-C irradiation

Inoculated verjuice samples were exposed to UV-C irradiation using a continuous-flow annular UV reactor system described in Kaya et al. (2015) and Unluturk and Atilgan (2014). UV reactor was made from annular quartz glass tube surrounded by a cylindrical aluminum reflector (Afe Olgunlar, Inc., Izmir, Turkey). It is equipped with seven UV-C lamps (UVP XX-15, UVP, Inc., Upland, CA). The lamps are 15 W low-pressure mercury arcs, which emit primarily at 253.7 nm. One UV-C lamp was positioned in the middle of the quartz tube, whereas the remaining six UV-C lamps were installed around the quartz tube. The gap size for annular flow was 5 mm. A heating jacket was used around the glass storage tank to control the temperature of the UV-C-treated liquid. A peristaltic pump (Watson Marlow, Inc., Cornwall, England) was utilized to pump the liquid from inlet through the outlet tube at different flow rates. The UV lamps in the system were switched on for about 15 min prior to UV treatment to minimize fluctuations in the intensity. The temperature of verjuice at the outlet of the UV system and inside the sample tank was checked by a K-type thermocouple (CEMDT-8891E, Shenzhen, China).

The verjuice (400 mL) was inoculated with acid adapted *S. cerevisiae* cells enriched in YPD broth and incubated at 30°C for 24 hr, which resulted in stationary-phase cultures containing approximately 10^6 and 10^4 CFU/mL. Thus, the effect of initial microbial load on the performance of the UV-C system was also evaluated. The same continuous flow UV reactor system was used for processing of different types of juice (lemon-melon juice blend, white grape juice, strawberry juice, etc.) aiming 5 log reductions in *Escherichia coli* K12 and spoilage microorganisms (Kaya et al., 2015; Kaya & Unluturk, 2016; Unluturk & Atilgan, 2014). The flow rate of the fruit juice and the number of cycles are selected in such a way that the discoloration and quality losses in fruit juice are minimized. The verjuice was circulated eight times with a flow rate of 3.80 mL/s through the reactor using 4 UV lamps as described in Kaya et al. (2015). The whole flow volume was collected in the glass sample tank at the end of one total cycle time during UV operation. Then, the next cycle was started. The UV exposure time and total processing time for one pass at 3.8 mL/s were manually recorded as 62 and 114 s, respectively. The average juice temperature in the system was recorded as 18.1 ± 2.2°C during process. The UV experiments were repeated three times and sampling was performed after each cycle. The UV dose delivered to the treatment medium was estimated by the potassium iodide/iodate actinometer (Rahn, 1997). The actinometrical buffer was pumped through the UV-C system at 3.8 mL/s. The increase in absorbance (352 nm) was measured at the outlet of the reactor after each cycle. Incident intensity (mW/cm^2) was calculated from the photon flux (254 nm; surface area of the annular reactor is 879.7 cm^2). The effective UV dose or fluence (J/cm^2) was calculated by multiplying the incident UV intensity and exposure time. Energy consumption per unit volume of the reactor (J/mL) was also calculated as described by Gayan et al. (2016) to compare the efficiency of the system with other systems in literature. The survival curves were obtained by plotting the logarithm of the survival fractions of *S. cerevisiae* (Log_{10} N/N_0) versus treatment doses (in units of J/cm^2). When UV radiation was combined with mild heat, the survival curves were also expressed in time unit (min) as used in the heat treatments.

2.4 | Mild heating

The lethal effect of heat on acid adapted *S. cerevisiae* in the verjuice was determined at two different mild heating conditions (MH1 and MH2) using the same UV-C reactor system (described in Section 2.3) by switching off UV lamps. For this purpose, water at 55 and 60°C in MH1 and MH2 treatment, respectively, was circulated around the jacket of the sample tank to keep the juice temperature constant in the tank. Preheated juice (400 mL, 50°C) was inoculated with acid adapted yeast culture, immediately put into the sample tank and circulated eight times through the heated system with a flow rate of 3.80 mL/s. The average temperatures of verjuice were recorded as 47.1 ± 1.4°C (MH1) and 50.5 ± 1.5°C (MH2) during processes. Sampling was performed after each cycle.

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2.5 | UV-C irradiation combined with mild heating (UV + MH)

UV-C irradiation process was employed by combining with two different mild heat treatments (UV + MH1 and UV + MH2). Preheated (50°C) juice was inoculated with acid adapted yeast culture and passed from the UV reactor system with a flow rate of 3.8 mL/s while UV-C lamps were switched on and the water at 55 and 60°C was being circulated throughout the system. The average temperatures of verjuice in the combined treatments were 48.3 ± 1.0°C (UV + MH1) and 51.3 ± 1.5°C (UV + MH2).

2.6 | Modeling of UV-C inactivation kinetics

Microorganisms in foods exposed by thermal and nonthermal treatments can exhibit different inactivation curves, such as linear, linear with tailing, linear with shoulder, sigmoidal-like included both shoulder and tailing, and biphasic (Geeraerd, Valdramidis, & Van Impe, 2005). If the number of microorganisms are reduced as a straight line with time, this is linear behavior. Thermal inactivation of microorganisms was conveniently explained by a log-linear model based on the first-order kinetics. The curve might have upward or downward concavity that caused by "shoulder" or "tailing" effect based on the microorganisms, treatment and medium characteristics. Shoulder effect (injury phase) may occur in the nonlethal UV dosage which is inert to the cells at the initial of the process; cells were exponentially died over this nonlethal dosage. Tailing effect may form at the end of the process in the case of different kind of microorganisms, suspended solids, improper mixing, agglomeration of killed cells, and different resistance of each cells (even if same species) to UV light. According to the presence of shoulder, tail, or both of them in the UV dose response curve, different kinetic models could be adjusted to the inactivation data of the microorganisms. Biphasic curve is similar to tailing effect and it occurs when existing two genetically different species are more resistant and more sensitive to UV light (Ferrario et al., 2015; Geeraerd et al., 2005; Lopez-Malo & Palou, 2005; Smelt & Brul, 2014).

GInaFiT (KU Leuven, Leuven, Belgium), a freeware tool program was used to test different types of microbial survival models for experimental data relating the change of the microbial population with time (Geeraerd et al., 2005). As survival curves of *S. cerevisiae* show shoulder, linear and tailing regions, log-linear (Equation 2; Van Boekel, 2002) and several nonlog-linear models, that is, log-linear plus shoulder (Equation 3; Geeraerd, Herremans, & Van Impe, 2000), log-linear plus tail (Equation 4; Geeraerd et al., 2000), and Weibull (Equation 5; Mafart, Couvert, Gaillard, & Leguerinel, 2002), were used to fit the inactivation data of *S. cerevisiae*.

$$\log\left(\frac{N}{No}\right) = -\frac{t}{D} \tag{2}$$

$$N = N_0 * e^{-k_{max}*t}\left(\frac{e^{k_{max}*SI}}{1+\left(e^{k_{max}*SI}-1\right)e^{-k_{max}*t}}\right) \tag{3}$$

$$N = (N_0 - N_{res}) * e^{-k_{max}*t} + N_{res} \tag{4}$$

$$\frac{N}{No} = 10^{-\left(\frac{t}{\delta}\right)^p} \tag{5}$$

where t is treatment time (s), D is the time needed for 90% inactivation of the cells, SI is the shoulder length time before exponential inactivation begins, k_{max} is inactivation rate constant of exponential death region, N_{res} is the residual number of subpopulations at the end of the treatment, p is the shape parameter describing downward concavity ($p > 1$) or upward concavity ($p < 1$) of the curve, and δ is the first decimal reduction time.

2.7 | Calculation of synergistic effect

The synergistic lethal effects of the combined treatments (UV + MH1 and UV + MH2) were calculated using Equation (7) (Gayan et al., 2012).

A 5-log reduction (99.999%) of the most resistant microorganisms in the juice was mandatory requirement of FDA. Theoretical 5D values ($5D_{UV+MH}$) were calculated by considering the individual contribution of UV ($5D_{UV}$) and MH ($5D_{MH}$) processes in Equation (6). $5D_{UV}$ and $5D_{MH}$ values were estimated from experimental data using the best fitted inactivation model. Experimental 5D values of combined treatments (UV + MH) were compared with the theoretical ones for prediction of the synergistic effect. If the inactivation effect of a combined process (UV + MH) was more than the individual inactivation effect of each process, the process would be synergistic. Otherwise, the hurdle process would be additive. Additive effect can be predicted from Equation (8) (Raso, Pagan, Condon, & Sala, 1998).

$$\text{Theoretical } 5D_{UV+MH} = \frac{(5D_{MH} * 5D_{UV})}{(5D_{MH} + 5D_{UV})} \tag{6}$$

$$\text{Synergism}\,(\%) = \frac{\text{theoretical } 5D_{UV+MH} - \text{experimental } 5D_{UV+MH}}{\text{theoretical } 5D_{UV+MH}} * 100 \tag{7}$$

$$D_{UV+MH} = 1/D_{UV} + 1/D_{MH} \tag{8}$$

2.8 | Statistical analysis

All experiments were repeated three times. Results of the logarithmic reductions were expressed as means and standard deviations. Error bars in figures correspond to the standard deviation of the mean. In order to determine goodness of the fits of models, the root mean square error (RMSE) values were compared. The over-fitting of the models was evaluated by calculating Akaike information criterion (AIC) and Bayesian Schwarz criterion (BIC). All RMSE, AIC, and BIC values were calculated from the equations given in the study of Carrillo et al. (2017). Both parameters are related to performance of the models, however, BIC value is more conservative with a harsher penalty. The smallest AIC and BIC values indicated the most accurate and parsimonious model (Quinn & Keough, 2002).

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3 | RESULTS AND DISCUSSION

3.1 | Characterization of the verjuice

Physicochemical and optical properties of verjuice were listed in Table 1. It is clear that the verjuice is a highly acidic juice due to low pH (pH: 2.63) and high acidity (TA: 2.89%) values. Hayoglu et al. (2009) also reported similar pH (2.98) and TA (2.48%) for verjuice obtained from Yediveren type of grapes. The slight difference in data between two studies can be attributed to the composition of grapes altered from area to area, because of different soil, location, and climate (Morris & Striegler, 2004). Microbial analyses showed that freshly squeezed verjuice had no background flora.

3.2 | UV treatments

The log reductions of *S. cerevisiae* after UV treatment were 0.40 ± 0.04 log CFU/mL and 0.54 ± 0.02 log CFU/mL for high (6.36 ± 0.04 log CFU/mL) and low (4.55 ± 0.09 log CFU/mL) initial loads, respectively (Figure 1). Applied UV dose in the treatment were calculated as 0.57 J/cm^2 (energy of 2.30 J/mL). The low initial load resulted in slightly higher inactivation compared to the high initial load of *S. cerevisiae*. Similarly, Kaya and Unluturk (2016) observed a low UV inactivation in white grape juice inoculated with high initial load of *S. cerevisiae*. The reason for this might be due to high number of microorganisms preventing the UV light to reach each microorganism found in the medium (Karel & Lund, 2003). Gouma et al. (2015) also studied the inactivation of *S. cerevisiae* STCC1172 in apple juice and obtained 0.51 log reduction of yeasts at 3.92 J/mL UV dose. Similarly, Keyser, Muller, Cilliers, Nel, and Gouws (2008) reported very low reduction (0.30 log) of yeasts and molds counts in turbid orange juice after exposure of 1.38 J/mL UV dose in the continuous flow UV reactor having 5 mm annular gap. However, Lopez-Malo, Guerrero, Santiesteban, and Alzamora (2005) observed higher than 3 log reduction of *S. cerevisiae* in clear apple juice by applying UV dose of 0.26 J/cm^2 (1.1 mW/cm^2 for 4 min) in the laminar flow system with 0.2 mm depth. Therefore, the low inactivation of the yeast cells in the present study was due to the high optical properties of verjuice (25.8 ± 0.1 cm^{-1}) and low UV penetration depth of juice layer flowing in 5 mm annular gap. The reason for low efficacy of the UV-C irradiation in this study might be also attributed to the suspended particles and laminar flow regime of the juice passing through the UV system (Fredericks, Du Toit, & Krugel, 2011; Koutchma et al., 2004). Koutchma et al. (2004) stated that particles in turbulent flow system provides better mixing and they are longer exposed to the UV-C light resulting better inactivation. Besides, it was reported that microorganisms naturally growing in juice products were more resistant to UV-C irradiation (Unluturk & Atilgan, 2014). The low level of inactivation in both conditions might be also attributed to the resistance of acid adapted yeast cells to UV-C light. Another reason of the low inactivation rate can be that yeast cells having larger size and different DNA structure than bacteria showing higher resistance to UV light (Bintsis et al., 2000; Tran & Farid, 2004). High inactivation efficiency of UV-C irradiation on several kinds of bacteria in fruit juices has been reported by many continuous flow studies (Caminiti et al., 2012; Kaya et al., 2015; Pala & Toklucu, 2011, 2013). Thus, hurdle strategies using mild heating in the UV-C system can be an alternative method to increase the efficiency of the UV-C system.

TABLE 1 Physicochemical and optical properties of freshly squeezed verjuice

Freshly squeezed verjuice			
pH		2.63	±0.02
TSS (°Bx)		4.29	±0.07
T.A. (%)		2.89	±0.01
Turbidity (NTU)		37.94	±2.01
Abs coef. (cm^{-1})		25.80	±0.05
Color	*L**	29.20	±0.06
	*a**	0.20	±0.00
	*b**	3.07	±0.02

Note: Results were presented as mean ± standard deviation (*n* = 3).
Abbreviations: TA, titratable acidity; TSS, total soluble solid content.

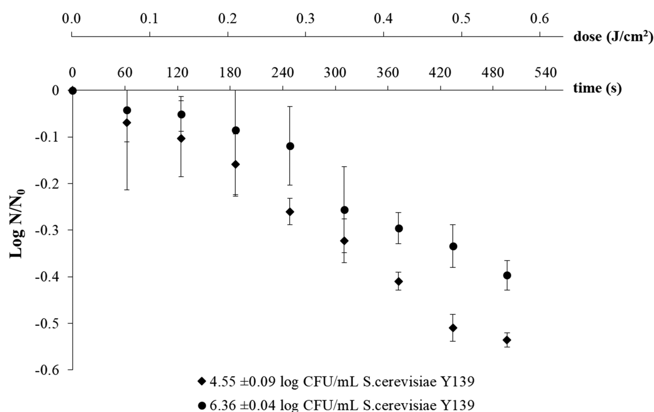

FIGURE 1 Influence of initial microbial density on *S. cerevisiae* inactivation in verjuice subjected to UV-C irradiation (error bars are standard deviation of the mean of three experiments)

3.3 | Mild heat treatments

MH1 (47.1°C) was resulted in 0.96 ± 0.25 log reduction for *S. cerevisiae* (Figure 2). However, MH2 (50.5°C) provided a higher inactivation, that is, 3.13 ± 0.05 log CFU/mL (Figure 3). This was attributed to the heating effect. Lopez-Malo, Guerrero, and Alzamora (1999) indicated that temperatures around 48–51°C are lethal for yeast vegetative cells with *D* values ranging from 10 to 30 min, which are comparable with the values obtained in this work (Table 3). The results of this study are in line with the findings of Gouma et al. (2015). They reported that heat inactivation of *S. cerevisiae* in apple juice was negligible up to 52.5°C requiring temperatures of at least 55°C for microbial heat inactivation. The maximum recorded temperature of verjuice in the MH2 treatment was 53.3°C. Thus, MH2 treatment was resulted in a decreased *D* value (Table 3). Additionally, it is known that a low pH value of the heating medium enhances microbial inactivation by decreasing the thermal

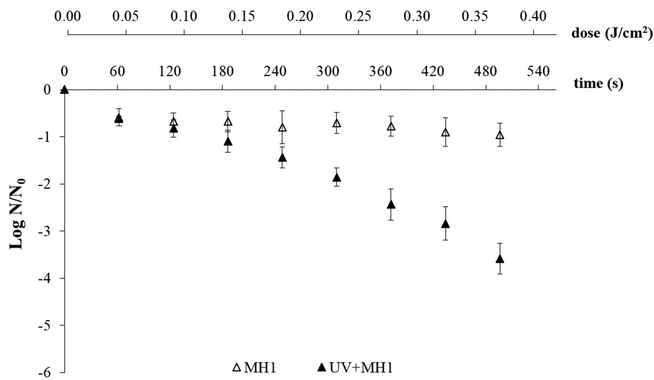

FIGURE 2 Inactivation of *S. cerevisiae* in verjuice subjected to mild heating alone (MH1) and its combination with UV-C irradiation (UV + MH1). Average verjuice temperatures were 47.1 ± 1.4°C for MH1 and 48.3 ± 1.0°C for UV + MH1 (Error bars are standard deviation of the mean of three experiments. The error bars that are smaller than the graph symbol are not displayed)

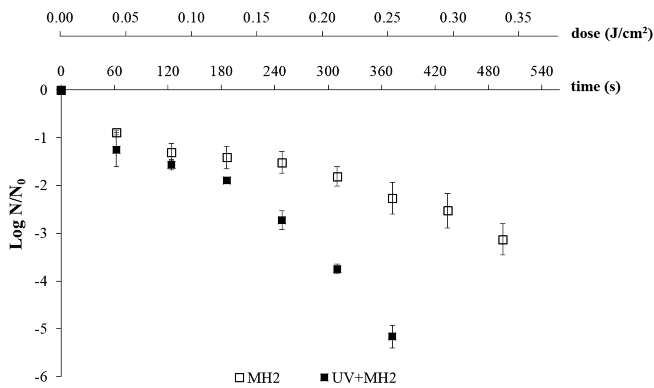

FIGURE 3 Inactivation of *S. cerevisiae* in verjuice subjected to mild heating alone (MH2) and its combination with UV-C irradiation (UV + MH2). Average verjuice temperatures were 50.5 ± 1.5°C for MH2 and 51.3 ± 1.5°C for UV + MH2 (Error bars are standard deviation of the mean of three experiments. The error bars that are smaller than the graph symbol are not displayed)

resistance of the microorganisms (Smelt & Brul, 2014). It was observed that heat inactivation of *S. cerevisiae* in verjuice significantly increased at temperatures above 50°C.

It was observed that MH treatments were not enough to meet the required pasteurization conditions of FDA (5D reduction). For this reason, these processes were combined with the UV-C irradiation to increase inactivation efficacy of the system.

3.4 | UV-C irradiation combined with mild heating (UV + MH)

The logarithmic reductions of the acid adapted *S. cerevisiae* in verjuice per each cycle during combined UV + MH treatments were shown in Figures 2 and 3. UV + MH1 treatment provided 3.59 ± 0.33 log CFU/mL reduction at 0.37 J/cm^2 dose (energy of 1.46 J/mL) and 48.3°C (Figure 2). However, the average temperature of verjuice was higher (51.3°C) for the second combination (UV + MH2) and yeasts were completely inactivated (5.16 ± 0.24 log CFU/mL) after 496 s (at the end of 6 cycles) with 0.25 J/cm^2 dose (energy of 1.01 J/mL; Figure 3). Gouma et al. (2015) found higher inactivation of yeasts by combining higher UV-C dose and mild heating at higher temperature. They obtained 1.3 log and >5-log reduction of *S. cerevisiae* by applying 2.9 J/mL at 55 and 60°C, respectively. However, Carrillo et al. (2017) found 3.5 log reduction of *S. cerevisiae* in carrot–orange juice blend with a UV dose of 10.6 kJ/m^2 at 50°C for 15 min. Gayan et al. (2014) reported similar reductions of *E. coli* O157:H7 in apple juice. They claimed 1.44, 2.86, and 5.47 log reduction of *E. coli* after 20.3 J/mL at 55, 57, and 60°C, respectively. At the same temperatures, 2.57, 3.41, and >6 log reduction of *E. coli* was achieved in orange juice after 13.6 J/mL (Gayan et al., 2012).

In conclusion, UV + MH2 treatment was able to achieve the US FDA's mandatory 5-log reduction in verjuice.

3.5 | Modeling of *S. cerevisiae* inactivation kinetics and synergistic lethal effect of combined UV + MH treatments

The survival curves did not show tails but rather exhibited shoulders except the survival curve of MH1. The inactivation kinetics of *S. cerevisiae* in verjuice subjected to UV, MH, and UV + MH treatments were best described by Weibull model with the smallest RMSE and AIC values (Table 2). According to Table 2, log-linear plus shoulder and log-linear plus tail models were not suitable to fit the inactivation data. BIC values of UV (high), MH1, MH2, and UV + MH1 treatments were the lowest for Weibull model. BIC values calculated for the linear and Weibull models fitting the data obtained in UV (low) and UV + MH2 treatments were not very different from each other (differences were less than 2). Dziak, Coffman, Lanza, and Li (2012) quoted that "the size of a difference in AIC or BIC between models is practically significant. For example, an AIC difference between two models of less than 2 provides little evidence for one over the other." Therefore, it was decided that the inactivation kinetics of *S. cerevisiae* were best described by Weibull model with the smallest RMSE and AIC values. Ferrario, Alzamora, and Guerrero (2013) also found that Weibull model was a good-fitted model for the sigmoidal inactivation behavior of *S. cerevisiae* in orange juice and natural apple juice exposed to pulsed light (included UV-C region) with very small AIC and BIC values. They also explained the slight variations in the inactivation curves could be a result of different resistance of the cells in a population to the applied stress. In the present study, there was a nonlinear fluctuation like a sigmoidal behavior in the inactivation curves of *S. cerevisiae* in verjuice after all treatments. Peleg and Cole (1998) stated that nonlinear curves may be generated from the cumulative lethal effect applied to the cells by time. Each individual cell can be killed at a specific time and the shape of the curves is depended on their inactivation distribution. Therefore, each of the acid adapted *S. cerevisiae* cells in the reactor might have a different resistance to UV or mild heat temperatures in the present study. Sigmoidal curves were more pronounced in UV treatments than in MH and UV + MH.

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TABLE 2 Goodness-of-fit (RMSE) and model overfitting (AIC, BIC) parameters for the log-linear, log linear plus shoulder, log linear plus tail, and Weibull models

	Log-linear			Log-linear + shoulder			Log-linear + tail			Weibull		
	RMSE	AIC	BIC	RMSE	AIC	BIC	RMSE	AIC	BIC	RMSE	AIC	BIC
UV (high load)	0.03	−53.34	−53.14	0.04	−51.95	−49.56	NS	NS	NS	0.03	−56.64	−54.24
UV (low load)	0.02	−60.4	−60.2	0.02	−59.02	−56.62	NS	NS	NS	0.02	−62.36	−59.96
MH1	0.17	−24.15	−23.95	NS	NS	NS	0.10	−32.82	−30.43	0.04	−51.95	−49.55
UV + MH1	0.18	−22.94	−22.74	0.19	−21.9	−19.5	NS	NS	NS	0.15	−26.74	−24.35
MH2	0.23	−18.69	−18.49	NS	NS	NS	NS	NS	NS	0.21	−21.2	−18.81
UV + MH2	0.36	−6.04	−6.09	0.40	−4.7	−2.8	NS	NS	NS	0.36	−6.04	−4.15

Note: GinaFiT NS reports for log-linear + shoulder model: "A negative value for the shoulder length Sl is physically not possible. Model is unlikely for this data" for log-linear + tail model "Log10(N_{res}) is less than the minimal measured value. Model with tailing is unlikely for these data."
Abbreviations: AIC, Akaike information criterion; BIC, Bayesian Schwarz criterion; NS, not suitable model; RMSE, root mean squared error.

TABLE 3 Thermal processing time for 5D pasteurization of verjuice based on Weibull model

	Delta (δ)	*p*	Log N_0 (log CFU/mL)	RMSE	*D* (min)	Experimental 5D (min)	Theoretical 5D (min)
UV (high load)	940.64	1.40	6.36	0.03	15.68	49.63	
(UV (low load)	819.53	1.16	4.55	0.02	13.66	54.73	
MH1	760.08	2.50	3.89	0.04	12.67	24.11	
UV + MH1	201.42	1.32	4.59	0.15	3.36	11.40	16.74
MH2	129.14	0.75	4.46	0.21	2.15	18.54	
UV + MH2	116.54	1.28	4.84	0.36	1.94	6.82	13.85

Abbreviation: RMSE, root mean squared error.

In other words, the inactivation curve of *S. cerevisiae* was closed to more linearity by applying mild heat treatment. All inactivation curves except MH2 demonstrated downward concavity ($p > 1$) according to the shape parameters of the Weibull model (Table 3). Therefore, yeast cells were not inactivated or they might be recovered themselves due to applying nonlethal UV dose or mild heating at the first cycles of the process (shoulder); then, they were exponentially reduced by the effect of cumulative lethal effect (linear). Similarly, Gouma et al. (2015) observed shoulder effect in the curves of yeast cells, which were able repair their DNA at the nonlethal UV dosages. There was a slightly noticeable tailing in the curves of UV treatments (Figure 1). The reason of tailing could be the presence of more resistant cells, residual suspended compounds such as accumulated death cells in the juice, or unequal distribution of UV dose in the reactor (Ferrario et al., 2013). Fitting of the Weibull model was also demonstrated by plotting the measured data from the experiments against the predicted data from the model (Figure 4). According to high R^2 values (0.95–0.99), Weibull model can be used to predict the inactivation of *S. cerevisiae* in verjuice treated by UV, MH or UV + MH treatments. Weibull model was also commonly used to explain the inactivation kinetics of the microorganisms in fruit juices exposed to UV-C irradiation. Lopez-Malo et al. (2005) reported that the inactivation curve of *S. cerevisiae* in apple juice exposed to UV-C irradiation was well explained by Weibull model with a high R^2_{adj} value (>0.98). Kaya and Unluturk (2016) also used the Weibull model (RMSE: 0.30) to the inactivation kinetics of *S. cerevisiae* in UV-C treated white grape juice at a UV dose of 136 mJ/cm^2. Both of these studies detected downward concavity ($p > 1$) in the curves, similar to the present study. Unluturk and Atilgan (2014) found that the inactivation of *E. coli* K12 and lactic acid bacteria in white grape juice by UV-C irradiation was best described by Weibull model with the smallest RMSE values (0.001 and <0.001). Baysal, Molva, and Unluturk (2013) studied the inactivation kinetics of *Alicyclobacillus Acidoterrestiris* in white grape juice exposed to UV-C treatment at 0.4 mW/cm^2 for 15 min. They found that Weibull model is suitable for describing the inactivation data with low RMSE (0.21).

In order to calculate the time needed for 5D pasteurization of verjuice, kinetic parameters of Weibull model (p, δ) were used (Table 3). Smallest *D* (1.94 min) and 5D (6.82 min) values were calculated from the combined UV + MH2 treatments. Additionally, downward concavity was detected since *p* value of this process was 1.28 ($p > 1$). This phenomenon can be observed when the nonlethal UV-dose was applied to resistant yeast cells at the initial period of the process. After that, yeasts cells could be further inactivated by the effect of heat. Similarly, Van Uden, Abranches, and Cabeca-Silva (1968) also estimated *p* as 1.5 for *S. cerevisiae* in a buffer solution (pH 3.5) after 50°C mild heat treatment. Complete inactivation of the cells (5.16 ± 0.24 log CFU/mL) was achieved with 6.2 min UV + MH2 treatment in the real experiment (Figure 3) while the experimental 5D value of this process was calculated as 6.82 min using the Weibull model parameters (Table 3). It could be said that the inactivation kinetics of the *S. cerevisiae* could be well explained by Weibull model.

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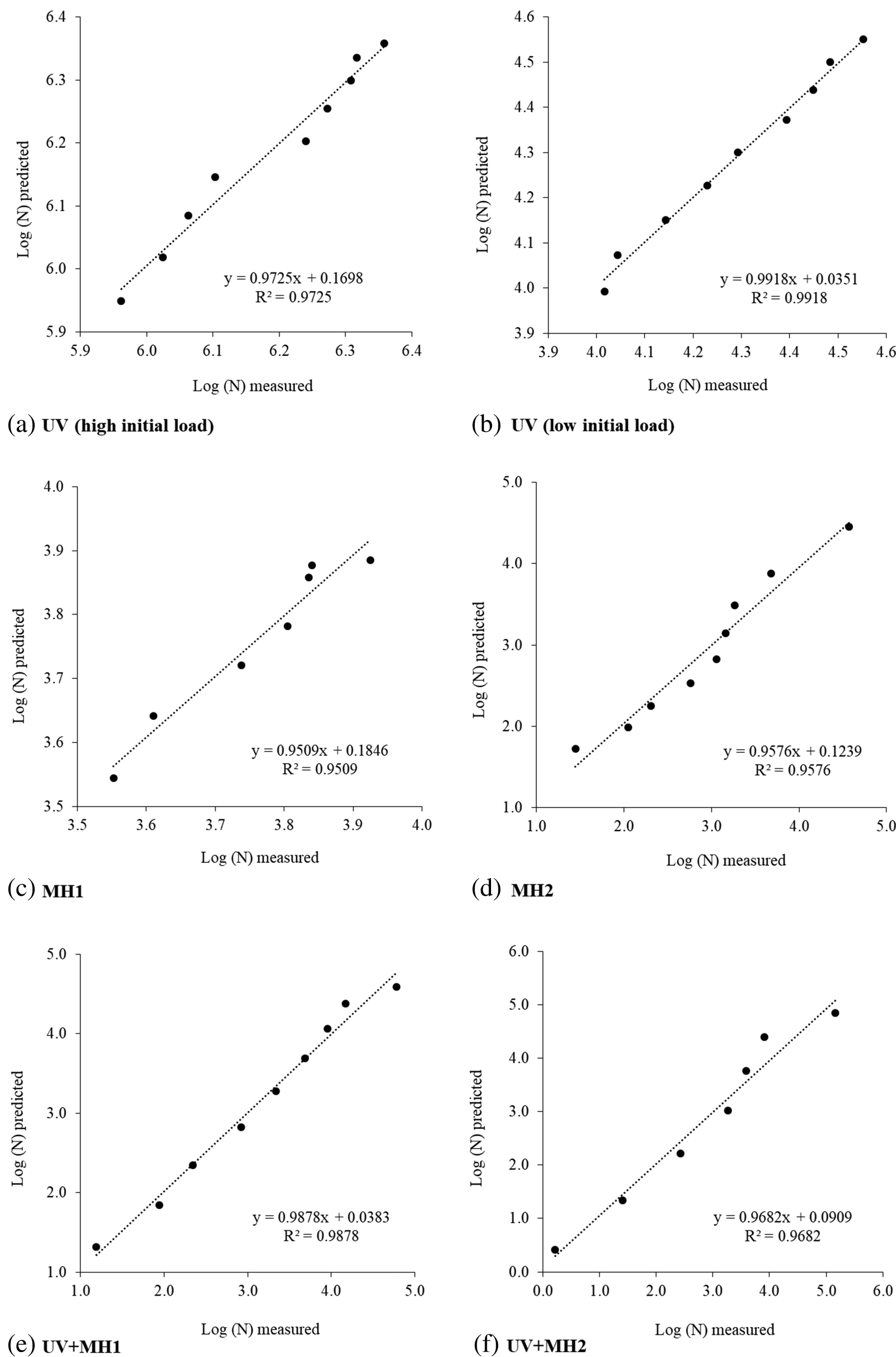

FIGURE 4 Relation between the measured data and predicted data obtained with the Weibull model for the inactivation of *S. cerevisiae* in verjuice subjected to UV, MH, and combined UV + MH treatments. (a,b) UV treatment with high and low initial load; (c,d) MH1 and MH2 treatments; (e,f) UV + MH1 and UV + MH2 treatments

The disinfection effect of UV light depends on absorption of the photons by DNA or RNA of the microorganisms causing lethal injury in the cells (Karel & Lund, 2003). On the other hand, microorganisms exposed to heat treatment can be inactivated by oxidation, protein denaturation, and membrane damage (Smelt & Brul, 2014). In addition to these individual inactivation mechanisms, synergistic lethality of the combined UV-C and mild heating could be also explained by the effect of heat inhibiting the DNA repair system of the cell which was previously damaged by exposure to UV-C irradiation. DNA could repair itself on the nonlethal dose of UV-C and low temperature (photoreactivation). However, further application of heat may cause irreversible damage on the DNA of the cell (Gayan et al., 2012). Results of combined processes indicate that there was a synergism between UV-C irradiation and mild heating when they were applied simultaneously rather than individually. In the first combination (UV + MH1), 31.90% synergism was calculated. This means that the *S. cerevisiae* cells were inactivated 31.90% times more in the combination of UV-C and mild heating than in the application of these processes individually. Furthermore, the synergistic lethal effect was calculated as 50.79% in the second combination (UV + MH2). The

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reason for higher synergistic effect could be explained by the dominant lethal effect of heat in the UV + MH2 (average juice temperature of 51.3°C). It was reported by several studies that synergism was affected by increase in temperature, however, it reached the highest value at 55°C (Gayan et al., 2013, 2014, 2016; Gouma et al., 2015). Gouma et al. (2015) observed that the lethal effect of UV light was significantly improved by heating at or above 50°C. They found maximum 33% synergism in the combination of UV and mild heating at 55°C. Similarly, Gayan et al. (2016) found the maximum synergism (35.1%) of UV + MH treatment at 52.5°C to inactivate *Salmonella typhimurium* in orange juice. Gayan et al. (2014) obtained 50% synergism by the combined treatment of UV-C (20.3 J/mL) and mild heating at 52.5°C on the inactivation of *Staphylococcus aureus* in apple juice. Gayan et al. (2013) reported that the synergistic lethal effect of UV + MH treatment was insignificant below 45°C for the inactivation of wild type *E. coli* in McIlvaine buffer, however, it was dramatically increased up to 55°C.

4 | CONCLUSIONS

In this study, the verjuice was characterized and the synergistic lethal effect of the UV-C irradiation and mild heat treatment on *S. cerevisiae* (NRRL Y-139), that is, the target spoilage microorganism in verjuice, was investigated. Complete inactivation (5.16 ± 0.24 log CFU/mL) of the yeast cells was achieved by combining UV light at 0.25 J/cm^2 dose (consumed energy of 1.01 J/mL) with mild heat treatment (MH2) at 51.3 ± 1.5°C. A synergistic lethal effect was detected when both technologies were applied simultaneously. Synergism of the combined process raised with an increase in temperature. The UV inactivation of *S. cerevisiae* was enhanced synergistically when the treatment temperature was increased above 50°C. This study leads to the possibility of designing combined methods for pasteurization of acidic fruit juices such as verjuice at moderate temperatures. Further research is required for investigating the shelf life of verjuice subjected to UV assisted mild heat treatment.

ACKNOWLEDGMENTS

This study was supported by the Department of Food Engineering, Izmir Institute of Technology (IZTECH-BAP, 2015-IYTE-08), and IZTECH Biotechology and Bioengineering Research and Application Center, Izmir, Turkey.

CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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How to cite this article: Kaya Z, Unluturk S. Pasteurization of verjuice by UV-C irradiation and mild heat treatment. *J Food Process Eng*. 2019;42:e13131. https://doi.org/10.1111/jfpe.13131

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