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Effectiveness of pulsed light treatments assisted by mild heat on *Saccharomyces cerevisiae* inactivation in verjuice and evaluation of its quality during storage

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ABSTRACT

The effects of pulsed light (PL) processing parameters such as depth of juice layer (1, 3, 5 mm), distance from the lamp (5, 10 cm) and number of pulses (0–50 pulses) on the inactivation of *Saccharomyces cerevisiae* in verjuice, a clarified beverage obtained from freshly-squeezed unripe grapes, were investigated. A reduction of 0.96 ± 0.27 log CFU/mL was achieved by applying a dose of 34 J/cm^2 (1-mm layer depth, 5-cm distance, 50 pulses). PL was combined with mild heating (MH) at 43, 45 and 47 °C to increase its inactivation efficacy. Pasteurization was achieved by applying 17 J/cm^2 at 45 °C (PLMH45–3) and 6.12 J/cm^2 at 47 °C (PLMH47–3) to a 3-mm juice layer with *S. cerevisiae* reductions of 5.10 ± 0.24 and 5.06 ± 0.08 log CFU/mL, respectively. Quality properties of PLMH47–3-pasteurized verjuice were monitored during 6 weeks of storage at refrigerated (5 °C) and room temperature (25 °C), The results were compared to those of untreated and thermally pasteurized (72 °C/18 s) samples. Untreated juice spoiled within 2 weeks at 25 °C. No growth was detected in other conditions for 6 weeks. Among quality characteristics, only optical properties changed slightly during storage. It was concluded that mild MH-assisted pulsed light treatments have potential for verjuice pasteurization compared to conventional thermal pasteurization due to the better preservation of its fresh-like characteristics.

1. Introduction

Verjuice (green juice, unripe grape juice) is an unfermented grape juice obtained by squeezing unripe table grapes. It has a specific aroma, flavour and sour taste (Aminian, Massoompour, Sadeg, & Omrani, 2003). Verjuice is generally used in Mediterranean and Southeastern Anatolia region as a savoury alternative to vinegar and lemon juice in traditional meals, vegetable salads and snacks to give flavour, and mixed as an ingredient in several alcoholic beverages and sauces (Oncul & Karabiyikli, 2015). Besides, it has antimicrobial and antioxidant properties (Aminian, Aminian, Nekooian, & Hoseinali, 2006; Karapinar & Sengun, 2007; Setorki, Asgary, Eidi, & Haeri Rohani, 2010). Verjuice is usually produced under household conditions that is not used any technological process. Thus, it is prone to microbial spoilage caused by yeasts and molds naturally growing in grapes (Hayoglu, Kola, Kaya, Özer, & Turkoglu, 2009). Several yeasts (*Pichia, Candida, Saccharomyces, Rhodotorula)* and molds (*Penicillium* sp., *Aspergillus* sp., *Eurotium*, *Alternaria*, *Cladosporium*, *Paecilomyces*, *Botrytis)* have been reported as spoilage microorganism in fruit juices (Aneja, Dhiman, Aggarwal, Kumar, & Kaur, 2014). Therefore, verjuice produced at household condition have been under the risk against spoilage and quality degradation.

Thermal pasteurization is traditionally used in fruit juices to inactivate pathogens and spoilage microorganisms, as well as enzyme spoilers. According to the Food and Drug Administration (FDA) regulation mandated by Hazard Analysis and Critical Control Point (HACCP) programme, “5-log reduction in the number of target microorganism is found to be necessary for fruit juice pasteurization” (U.S. FDA, 2001). However, the pasteurization term was re-defined and extended by the USDA National Advisory Committee on Microbiological Criteria for Foods (NACMCF) as “Any process, treatment, or combination thereof that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage” (NACMCF, 2006). The thermal treatment in juice processing is principally performed at 65 °C for 30 min, 77 °C for 1 min and

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88 °C for 15 s (Miller & Silva, 2012). However, these treatments may lead to important chemical and physical changes that impair organoleptic properties, degrade nutrients and reduce the content of some bioactive compounds (Gómez, Welti-Chanes, & Alzamora, 2011; Tiwari, O'Donnell, & Cullen, 2009; Wang, Guo, Ma, Zhao, & Zhang, 2018). Therefore, the application of mild thermal treatments in combination with other technologies is sought by fruit juice processors in order to maintain the taste of the product while preserving their nutritional value (Vervoort et al., 2011). Besides, alternative nonthermal technologies have been developed in order to fulfill the demands of consumers and prevent the undesirable effects of thermal pasteurization (Raso & Barbosa-Cánovas, 2003).

Pulsed light (PL) technology is used as a nonthermal processing method to eliminate pathogenic and spoilage microorganisms in foods. It is applied to solid and liquid foods by using short time (1 μs to 0.1 s) high-peak pulses between 200 and 1100 nm (Barbosa-Cánovas, Pothakamury, Palou, & Swanson, 1998; Oms-Oliu, Martín-Belloso, & Soliva-Fortuny, 2010). PL can be described as a multi-target inactivation process with photochemical (cellular DNA damage), photothermal (cell overheating) and photophysical (cell membrane destruction) effects being involved (Gómez-López, Ragaert, Debevere, & Devlieghere, 2007; Krishnamurthy, Demirci, & Irudayaraj, 2007). It has been reported that the UV-C region (200–280 nm) has a great impact on the inactivation of microorganisms (Gómez-López et al., 2007; Koutchma, 2008). FDA has approved the use of PL on foods and food surfaces under strict conditions (U.S. FDA, 1996). Some critical factors affect the performance of PL, including the distance from the lamp, sample thickness, contamination level of sample and sample composition (Gómez-López et al., 2007). Studies on PL indicate that this technology could be a promising alternative method for the decontamination of several solid foods, such as meat products (Ganan, Hierro, Hospital, Barroso, & Fernández, 2013), vegetables (Gómez-López, Devlieghere, Bonduelle, & Debevere, 2005) and fruits (Koh, Noranizan, Karim, & Nur Hanani, 2016), as well as liquid foods, such as fruit juices (Ferrario, Alzamora, & Guerrero, 2013; Maftei, Ramos-Villarroel, Nicolau, Martin-Belloso, & Soliva-Fortuny, 2014; Sauer & Moraru, 2009). PL technology can also be combined with other technologies (ultrasound, thermosonication, mild heating, etc.) to increase its antimicrobial effect in foods (Ferrario, Alzamora, & Guerrero, 2015; Hilton, de Moraes, & Moraru, 2017; Muñoz et al., 2011).

The main objective of this study was to evaluate the efficacy of the PL technology to extend the shelf-life of verjuice with a maximum quality. The first part of this work pertained to the application of PL treatments alone or in combination with mild heat conditions. In this part, the effect of various PL processing parameters, such as the depth of juice layer, distance from the lamp and the number of pulses on the logarithmic reduction of a target microorganism in verjuice, i.e. *Saccharomyces cerevisiae* (NRRL Y-139), were investigated. In the second part, several quality properties of PL-pasteurized verjuice were monitored and compared to those of thermally pasteurized and untreated verjuice samples throughout storage. This study could add novel information to the literature because it could not be found any study on the inactivation of yeasts in fruit juices by using combined pulsed light and MH treatments as a hurdle method. Besides, traditionally produced verjuice can be industrially pasteurized and market by using the data of this study.

2. Materials and methods

2.1. Preparation and characterization of verjuice

Freshly squeezed verjuice was prepared from Yediveren variety of unripe grapes (*Vitis vinifera L.*) purchased from vineyards in Urla region of Izmir, Turkey. Unripe grapes were harvested based on their maturity index (Eq. 1) (Palomo, Díaz-Maroto, Viñas, Soriano-Pérez, & Pérez-Coello, 2007).

Table 1
Physicochemical characteristics of untreated verjuice.

Measurement		Value*	
pH		2.58	± 0.01
Total soluble solids (°Brix)		3.70	± 0.00
Titratable acidity (%)		1.99	± 0.03
Turbidity (NTU)		27.20	± 0.71
Absorption coeffient (cm^{-1})		19.79	± 0.00
CIE Color parameters	L*	35.24	± 0.44
	a*	0.26	± 0.09
	b*	4.36	± 0.27
	h° ($\tan^{-1}(b*/a*)$)	86.63	± 1.04
	C* ($a^{*2} + b^{*2})^{1/2}$	4.37	± 0.28

* : Values are the mean of three independent determinations ± standard deviation.

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

Detartarisation was applied to freshly squeezed verjuice by keeping the juice refrigerated (5 °C) for 1 day to precipitate tartrate particles. After filtering, clear verjuice was thermally pasteurized (72 °C/18 s) in order to eliminate background biota before inactivation studies.

Physicochemical properties of verjuice were determined before PL treatment. The pH, total soluble solid content (°Brix), absorption coefficient (cm^{-1}) and turbidity (NTU) of verjuice were measured using a bench-top pH meter (Crison Instruments SA, Barcelona, Spain), a hand-held refractometer (Palette PR-32, Atago USA, Inc.), a spectrophotometer (Jasco V-650, Jasco Europe Srl, Italy) and a portable turbidimeter (HI 98703, Hanna Instruments, Inc., USA), respectively. The CIE color parameters L^*, a^*, b^*, were measured directly using a Minolta CR-400 chromameter (Konica, Inc., Japan). Physicochemical characteristics of verjuice are shown in Table 1. Additionally, hue angle (h°) and chroma (C^*) values were calculated according to equations displayed in Table 1.

2.2. Microbiological analysis

2.2.1. Background microbiota

Total mesophilic aerobic bacteria (TMAC), yeasts and molds (YMC), and total coliforms (TC) of verjuice were counted by spread-plating on Plate Count Agar (PCA, Merck, Darmstadt, Germany), Potato Dextrose Agar (PDA, Difco Laboratories, Detroit, Mich) acidified to pH 3.5 with 10% tartaric acid (Merck, Darmstadt, Germany) and Violet Red Bile Agar (VRBA, Merck, Darmstadt, Germany) plates, respectively. PCA, PDA and VRBA plates were then incubated at 30 °C for 48 h, at 25 °C for 2–5 days and at 37 °C for 24 h, respectively. No background microorganisms (TMA, YMC and TC) were detected in verjuice.

2.2.2. Target microorganism and acid adaptation study

Verjuice was initially fermented in order to decide on a dominating strain with spoilage potential that would be used in further inoculation studies. For this purpose, freshly squeezed verjuice was obtained from the unwashed berries and placed in a shaker (Thermo Electron Corp., Ohio, USA) and shook at 200 rpm and 30 °C for a couple of days. At the end of natural fermentation trials, several molds and yeasts species were detected in the verjuice samples. On the basis of the results, yeasts were the main microorganism causing spoilage of verjuice. Thus, *Saccharomyces cerevisiae* (NRRL Y-139) was selected as the target microorganism in verjuice to evaluate the efficiency of the processes.

Stock cultures of *S. cerevisiae* containing 25% glycerol were stored at −80 °C at University of Lleida before use. *S. cerevisiae* was cultured in yeast extract-peptone-dextrose (YPD) broth (manually prepared from 10 g yeast extract, 20 g peptone and 20 g dextrose per 1 L distilled water). *S. cerevisiae* colonies were spread on acidified PDA plates (pH 3.5) and enumerated after incubation at 30 °C for 48 h. Stock

cultures were enriched in a flask containing Yeast-Extract-Peptone-Dextrose (YPD) broth and incubated overnight (24 h) at 30 °C and 200 rpm before inoculation.

S. cerevisiae cells did not survive in the acidic conditions of verjuice after direct inoculation from the stock culture. Thus, cells were first adapted to high acidic conditions by gradually reducing the pH of the YPD broth with the addition of 10% tartaric acid. Firstly, 100 μL of the stock culture of *S. cerevisiae* was inoculated in 100-mL YPD broth (pH 3.5) and incubated in a shaker at 200 rpm and 30 °C for 24 h. After incubation, 1 mL of enriched cells was transferred to the YPD broth at pH 2.7, and incubated in the shaker for 48 h under the same conditions (30 °C, 200 rpm) until cell counts reached 10^6 CFU/mL. The acid-adapted yeast cells were inoculated into verjuice and enumerated on PDA at several different time intervals during incubation (30 °C, 200 rpm). It was observed that the number of viable yeast cells did not significantly change in the highly acidic conditions of verjuice during incubation for 6 h. The acid-adapted *S. cerevisiae* cells were then collected and plated on the PDA slants (acidified to pH 3.5 with 10% tartaric acid), incubated at 30 °C for 2 days and then stored at 5 °C before use in microbial inactivation experiments.

2.3. Processing of verjuice

Verjuice samples were inoculated with acid-adapted *S. cerevisiae* cells with an initial load of ~10^4–10^5 CFU/mL (4–5 log CFU/mL) to mimic "the worst-case scenario" occurring in a spoiled product. Then, inoculated verjuice samples were exposed to pulsed light (PL), mild heating (MH) and to a combination of PL and MH treatments (PLMH), separately, to determine the best processing conditions ensuring 5 log reductions of *S. cerevisiae*, as proposed by Gabriel (2012). All the processes and experimental conditions used in this study are listed in Table 2.

2.3.1. Pulsed light treatments

Verjuice inoculated with acid-adapted *S. cerevisiae* was exposed to PL by using an automatic laboratory-scale bench-top XeMaticA-2 L System (SteriBeam Systems GmbH, Germany). The device consists of a standard air-cooled 18-cm long UV-C transparent quartz xenon lamp located above the device, a stainless-steel table, a chamber door, and power and control panels. The emitted spectrum in the system ranged from 180 to 1100 nm, with 15–20% of the light in the UV region. Samples were placed on a shelf situated 5 or 10 cm below the light source and were irradiated with flashes of a duration of 0.3 ms with energy densities of 0.68 and 0.34 J/cm^2/pulse.

For PL processing, verjuice was poured into a 5 cm-diameter petri dish and inoculated with acid-adapted *S. cerevisiae*. Different depths of verjuice layer (1, 3, or 5 mm) and distance from the xenon lamp (5 or 10 cm) were assayed. The samples were exposed to up to 50 pulses and stirred once every 10 pulses to prevent overheating. Lethal heating did not occur during the treatment as temperature was under 35 °C. The overall PL dose after treatments was calculated as 34 J/cm^2 and 17 J/cm^2 for a distance of 5 and 10 cm, respectively.

In order to calculate fluence in the system, energy calculation was firstly carried out by using a photodiode detector located at different distances (from 4 to 14 cm) from the lamp. Then, an oscilloscope was connected to the photodetector and the obtained signals converted to radiance values by using a standard light source and a calibration curve drawn according to the manufacturer's instructions. The exponential equation of the curve plotted between the fluence and distance data was found as $y = 3.3743 \times^{-1.004}$, where x is the distance (cm), and y is the fluence (J/cm^2). This equation was used to calculate the corresponding fluence at a desired distance. Fluence values for the sample surfaces located 10 and 5 cm away from the lamp were calculated as 0.34 and 0.68 J/cm^2/pulse, respectively. The total PL dose (J/cm^2) applied to verjuice was determined by multiplying the number of pulses of each treatment by the fluence per pulse.

2.3.2. Mild heating treatments

A glass-jacketed beaker (3, 5 mm juice layer) connected to a temperature-controlled water bath was used. The internal temperature of verjuice in the beaker was adjusted to 43, 45 and 47 °C (Table 2) and kept constant by controlling with a K-type thermocouple. Verjuice inoculated with *S. cerevisiae* was subjected to MH treatments: 43 °C for 20 min, 45 °C for 20 min and 47 °C for 8.5 min, respectively (yeast counts were below the detection limit beyond 8.5 min at 47 °C). Samples taken at specific intervals during the MH treatments were rapidly

Table 2
Experimental design of PL, MH and combined treatments.

Treatment	Experiment	Treatment code	Juice layer depth (mm)	Distance from the lamp (cm)	Dose (J/cm^2)
PL	1	PL5–1	1	5	0, 6.8, 13.6, 20.4, 27.2, 34
	2	PL10–1	1	10	0, 3.4, 6.8, 10.2, 13.6, 17
	3	PL5–3	3	5	0, 6.8, 13.6, 20.4, 27.2, 34
	4	PL10–3	3	10	0, 3.4, 6.8, 10.2, 13.6, 17
	5	PL5–5	5	5	0, 6.8, 13.6, 20.4, 27.2, 34
	6	PL10–5	5	10	0, 3.4, 6.8, 10.2, 13.6, 17

Treatment	Experiment	Treatment code	Juice layer depth (mm)	Temperature	Treatment time (min)
MH	1	MH43–3	3	43	20
	2	MH45–3	3	45	20
	3	MH47–3	3	47	8.5
	4	MH43–5	5	43	20
	5	MH45–5	5	45	20
	6	MH47–5	5	47	8.5

Treatment	Experiment	Treatment code	Juice layer depth (mm)	Temperature	Dose (J/cm^2)
PLMH*	1	PLMH43–3	3	43	0, 3.4, 6.8, 10.2, 13.6, 17
	2	PLMH45–3	3	45	0, 1.7, 3.4, 5.1, 6.8, 10.2, 13.6, 17
	3	PLMH47–3	3	47	0, 1.02, 2.04, 3.06, 4.08, 5.10, 6.12
	4	PLMH43–5	5	43	0, 3.4, 6.8, 10.2, 13.6, 17
	5	PLMH45–5	5	45	0, 1.7, 3.4, 5.1, 6.8, 10.2, 13.6, 17
	6	PLMH47–5	5	47	0, 1.02, 2.04, 3.06, 4.08, 5.10, 6.12

PL: Pulsed-UV light. MH: Mild Heating. PLMH: Pulsed-UV light combined with mild heating. * distance from the lamp was 10 cm in PLMH treatments.

cooled in an ice bath. The efficacy of MH treatment on the inactivation of *S. cerevisiae* in verjuice was then evaluated by enumerating the *S. cerevisiae* colonies on PDA using a spread plating technique before and after the application of the MH treatments.

2.3.3. Pulsed light treatments assisted with mild heating

The combined treatment (PLMH) was studied as a hurdle technology approach. Verjuice was poured into the jacketed beaker used in MH treatment, placed into the PL treatment chamber and connected to the water bath. The verjuice temperature was adjusted to 43, 45 and 47 °C and checked with a K-type thermocouple. Next, the verjuice inoculated with *S. cerevisiae* and placed 10 cm away from the lamp was exposed to simultaneous PL and MH treatment (Table 2). The total number of pulses in the combined treatments was adjusted by considering the overall exposure time to the MH conditions. Samples were removed at specific pulse intervals and immediately cooled in an ice bath. The *S. cerevisiae* counts in the verjuice samples were evaluated by spread plating on PDA.

2.4. Evaluation of verjuice quality and shelf-life through storage

Shelf-life of pasteurized verjuice samples was evaluated by monitoring microbiological, physicochemical, optical, and antioxidant activity properties under refrigerated (5 °C) and room temperature (25 °C) conditions for 6 weeks.

Freshly squeezed verjuice was pasteurized using the PLMH47–3 treatment (6.12 J/cm^2 dose + 47 °C for 8.5 min), hence achieving 5 log reductions of *S. cerevisiae*. Thermally pasteurized (P) and untreated (U) verjuice samples were used as positive and negative controls. Conventional thermal pasteurization (72 °C for 18 s) was also applied to verjuice by using a continuous system composed of a stainless-steel capillary tube, a pump, a thermostatic bath (Huber GmbH, Offenburg, Germany) and a cooling cabinet (adjusted to 5 °C). The pasteurized and cooled verjuice was collected into a 1 L sterile glass bottle and used for the shelf-life study.

Approximately 25–30 mL of the untreated and pasteurized verjuice samples were transferred to sterile dark glass bottles (50 mL) and stored under refrigerated and room temperature conditions for 6 weeks, with periodic sampling at 0, 1, 2, 4 and 6 weeks. The temperatures were checked during storage at specific time intervals.

All verjuice samples were analysed for microbiologically by counting total aerobic mesophilic count (TAMC), yeasts and molds count (YMC) and total coliforms (TC), physicochemical (pH, TSS content, TA), optical (absorption coefficient, turbidity, color) and the antioxidant (DPPH· scavenging activity) attributes. Physicochemical and optical properties were determined as outlined in section 2.1. Total color difference (ΔE) and browning index (BI) were also calculated using Eq. (2) and Eq. (3), respectively.

$$\Delta E = \sqrt{(\Delta L*)2 + (\Delta a*)2 + (\Delta b*)2} \quad (2)$$

$$\mathrm{BI} = 100 * \frac{\frac{(a* + 1.75\,L*)}{(5.645\,L* + a* - 3.012\,b*)} - 0.31}{0.172} \quad (3)$$

The antioxidant activity of verjuice samples was investigated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method proposed by Pataro, Sinik, Capitoli, Donsì, and Ferrari (2015) with slight modifications. A reagent solution consisting of 3.9 mL of daily-prepared DPPH methanolic solution (25 ppm), 10 μL verjuice sample and 90 μL deionized water were placed in plastic cuvettes and the mixture was kept in the dark at 20 °C for 60 min. The blank sample was composed of 3.9 mL DPPH solution and 100 μL deionized water instead of verjuice. Autozero was done with methanol. Absorbance of the cuvettes was measured at 515 nm. The antioxidant activity was referred to the DPPH radical inhibition (%) as defined by Eq. (4), where the absorbance of the sample (after 60 min) and blank were identified as A_s and A_b, respectively.

$$DPPH\ inhibition\ (\%) = 100 - \left(\frac{A_s}{A_b} x100 \right) \quad (4)$$

2.5. Statistical analyses

All the experiments carried out in this work were repeated thrice. Regression analysis was applied by using a commercial spreadsheet (Microsoft Excel, Redmond, WA, USA). One-way analysis of variance (ANOVA) was done to determine how significantly the independent variables (treatments) affected the changes in the dependent variables (quality properties) during the shelf-life of verjuice. The means of data were compared by Tukey's pairwise comparison test at a 95% confidence interval ($p \leq 0.05$). Minitab 16 software program (Minitab, Inc., State College, PA, USA) was used for the one-way ANOVA and LSD test of the results.

3. Results and discussion

3.1. Effect of PL, MH and their combination on the inactivation of S. cerevisiae in verjuice

PL had little lethal effect on *S. cerevisiae* cells in verjuice under studied conditions (Fig. 1). The depth of juice layer and the distance from the lamp, thus the amount of incident energy, was found to be the most critical factor affecting the efficiency of PL process in this study. The highest reduction (0.96 ± 0.27 log CFU/mL) was obtained when delivering a dose of 34 J/cm^2 in a 1-mm layer of juice sample placed 5 mm away from the lamp [PL5–1] (Fig. 1a). Besides, for a same distance from the lamp (5 cm) and irradiance (34 J/cm^2), the reductions of *S. cerevisiae* in 3 mm-deep [PL5–3] and 5 mm-deep [PL5–5] juice layers were significantly lower (0.35 ± 0.03 log CFU/mL and 0.52 ± 0.01 log CFU/mL, respectively) without significant differences between them ($p > 0.05$). It has been reported that 90% of the light can pass through only the first 1 mm layer of the juices (Keyser, Muller, Cilliers, Nel, & Gouws, 2008; Sizer & Balasubramaniam, 1999). On the other hand, for a distance to the lamp of 10 cm, only 0.60 ± 0.03 log CFU/mL reduction was achieved for a fluid depth and UV irradiance of 1 mm [PL10–1] and 17 J/cm^2 (Fig. 1b) ($p \leq 0.05$). The impact of PL technology on the inactivation of acid-adapted *S. cerevisiae* strains in verjuice of different depths and distances from the xenon lamp was also summarized in Table 3. According to Table 3, there was no statistical difference between the microbial reduction in verjuice having 3 mm and 5 mm depth exposed to PL at 5 cm distance. However, the number of the cells were significantly reduced in the 1 mm verjuice depth exposed at the same distance ($p \leq 0.05$). Similarly, there was a significant difference between the *S. cerevisiae* reduction in verjuice having 1 and 5 mm depth 10 cm distance ($p \leq 0.05$). Therefore, the depth of juice layer was an important factor in this PL system. In the verjuice having 1 mm depth, the reduction of *S. cerevisiae* at a distance of 5 cm was significantly greater compared to 10 cm distance ($p \leq 0.05$). However, the distance was not a key parameter in the inactivation efficacy of the PL system when using 3 and 5 mm depths of verjuice. The reason for the reduced efficacy of the PL system could be the less dose calculated for the 10 cm distance (17 J/cm^2) relative to the 5 cm distance (34 J/cm^2) from the xenon lamp. Similarly, Artíguez, Lasagabaster, and de Marañón (2011) and Said, Ben, Federighi, Bakhrouf, and Orange (2010) indicated that a high microbial inactivation in the liquids could be achieved with a high PL dose, which could be obtained by reducing the sample depth and the lamp distance and by increasing the number of the pulses. Maftei, Ramos-Villarroel, Nicolau, Martin-Belloso, & Soliva-Fortuny, 2014 found 3.76 log CFU/mL reductions of *Penicillium expansum* spores in clear apple juice having 8 mm depth after PL dose of 32 J/cm^2. The higher inactivation rates achieved than this study even if

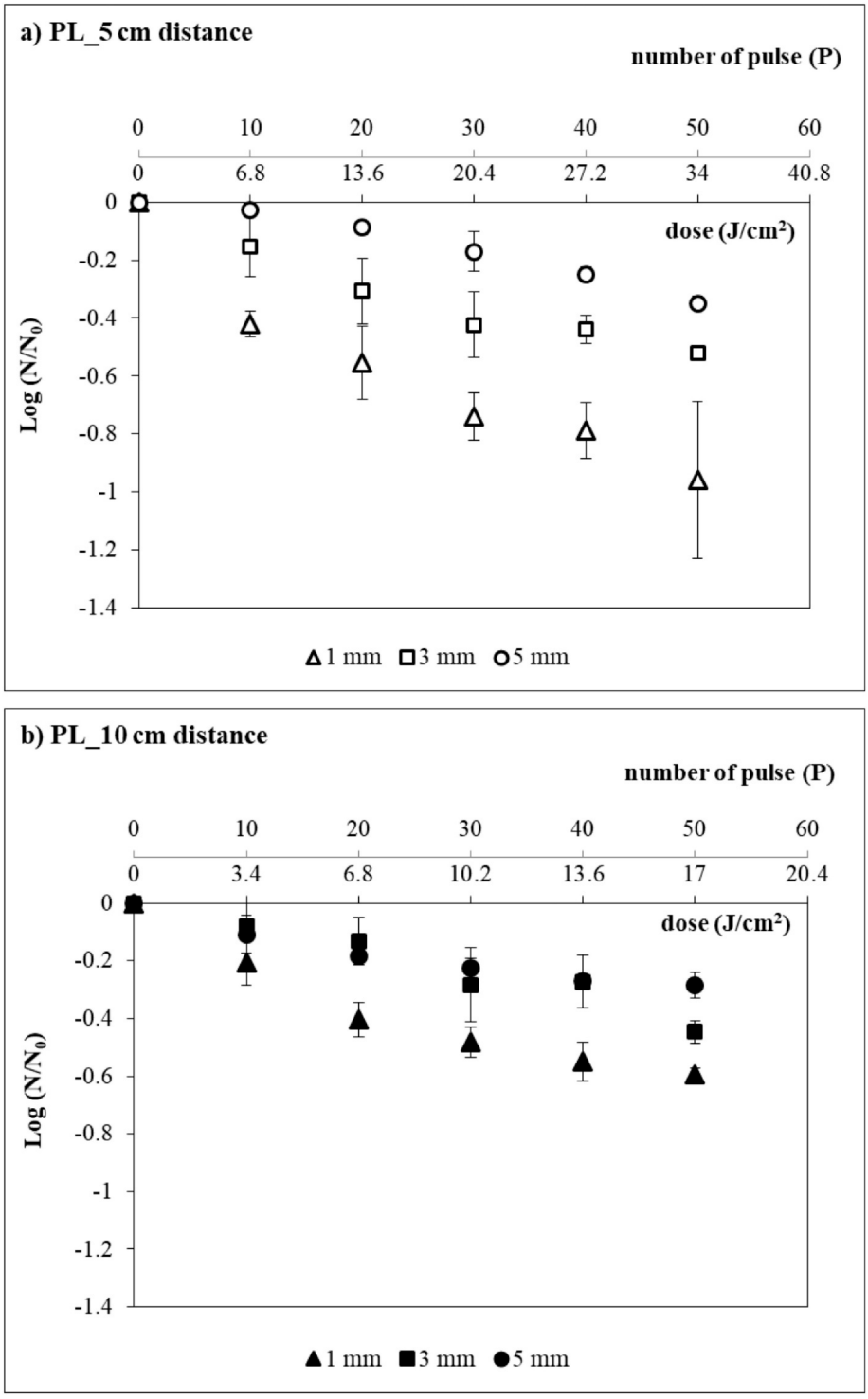

Fig. 1. The inactivation of acid-adapted *S.cerevisiae* (NRRL Y-139) in verjuice by PL treatment at different lamp distance 5 cm (a) and 10 cm (b).

they exposed PL to mould spores in their study. This can be likely a consequence of the high UV light penetration rate in the clarified apple juice with less turbidity and absorbance values than freshly squeezed verjuice. Ferrario, Alzamora, & Guerrero, 2013 observed that the inactivation of *S. cerevisiae* in natural apple juice (< 1 log CFU/mL) was significantly lower than in the clarified juice (~4 log CFU/mL) when applying a PL dose of 71.6 J/cm^2. It was stated that the light absorptivity depends on liquid properties, such as suspended particles, color and TSS (Aguirre, Hierro, Fernandes, & de Fernando, 2014; Keyser et al., 2008). It was reported that *S. cerevisiae* cells were highly resistant to UV-C wavelengths, due to their larger size and different DNA structure compared to bacteria (Fredericks, du Toit, & Krugel, 2011; Tran & Farid, 2004). The PL resistivity of *S. cerevisiae* in this study could also be a result of using acid-adapted cells. These cells might have an altered protein expression and membrane lipid composition that could alleviate the PL effects on the DNA (Huang, Tsai, & Pan, 2007; Yuk & Marshall, 2004).

All MH and hurdle treatments (PLMH) based on depth of juice layer at different temperatures were demonstrated in Fig. 2. Considering MH treatments, the maximum reduction of the acid-adapted *S. cerevisiae*

Table 3
Log reductions of acid-adapted *S.cerevisiae* (NRRL Y-139) inoculated into verjuice by PUV treatment at different lamp distances and juice layer thickness.

Treatment code	Lamp distance (cm)	Depth of juice Layer (mm)	Log reduction (log No/N)	PUV dose (J/cm^2)
PUV5–1	5 cm	1 mm	0.96 ± 0.27a	34
PUV5–3	5 cm	3 mm	0.52 ± 0.01bc	34
PUV5–5	5 cm	5 mm	0.35 ± 0.03bc	34
PUV10–1	10 cm	1 mm	0.60 ± 0.03b	17
PUV10–3	10 cm	3 mm	0.45 ± 0.04bc	17
PUV10–5	10 cm	5 mm	0.28 ± 0.05c	17

Results were presented as "means ± standard error". The least significant difference was determined by Tukey pairwise comparison test. Means that do not share the same letter are significantly different ($p \leq 0.05$).

cells (3.4 ± 0.44 log CFU/mL) was achieved at 47 °C for 8.5 min [MH47] (Fig. 2c). In contrast, 2.28 ± 0.36 and 1.35 ± 0.29 log CFU/mL reductions were obtained after treating at 45 and 43 °C for 20 min [MH45 and MH43], respectively (Fig. 2a & 2b). All MH inactivation curves showed upward concavity with a tailing phase. García Carrillo, Ferrario, and Guerrero (2018) observed the same trend for *S. cerevisiae* in a carrot-orange juice blend at 50 °C and suggested the tailing resulted from the presence of heat-resistant cells in the population. The high inactivation for mild heating at 47 °C was due to the lethal effect of heat on the yeast cells. Furthermore, it is known that a low pH value of the heating medium enhances microbial inactivation by decreasing the thermal resistance of microorganism (Smelt & Brul, 2014). Gouma, Gayán, Raso, Condón, and Álvarez (2015) observed ~0.2 and 0.5 log CFU/mL reduction of *S.cerevisiae* STCC 1172 in apple juice (pH: 3.6) after 3.5 min at 50 and 55 °C, respectively. Treatment of verjuice at 47 °C for 3.5 min resulted in a comparatively higher reduction (2.4 log CFU/mL) of acid-adapted *S. cerevisiae*. Similarly, 2.6 log CFU/mL reductions of *S. cerevisiae* (KE162) were achieved in carrot-orange juice blend (pH: 3.8, 9.8°Brix) at 50 °C for 15 min (García Carrillo et al., 2018). In this study, a similar reduction (2.6 log) was attained for acid-adapted *S. cerevisiae* by heating verjuice at 45 and 47 °C for the same treatment time. Gabriel (2012) reported 1 log reduction in the number of *S. cerevisiae* cells (BFE-39) in apple juice (pH: 3.7, 12.5°Brix) exposed to 55 °C for 6.2 min. A justification for the relatively higher reduction of *S. cerevisiae* at lower MH temperatures or shorter time in the current study could be attributed to the lower acidic conditions (pH 2.6) and lower sugar content (3.7°Brix) of verjuice. Shearer, Mazzotta, Chuyate, and Gombas (2002) pointed out that the heat resistance of *S. cerevisiae* in various juices depends on the composition, as well as on pH. Several studies also state that the heat-resistant of yeast cells in juices increased with an increase in the soluble solids content (Beuchat, 1982; Juven, Kanner, & Weisslowicz, 1978; Torreggiani & Toledo, 1986).

The log reductions of acid-adapted *S. cerevisiae* in verjuice exposed to combined (PLMH) treatments are also shown in Fig. 2. Juice layer depth and treatment temperature were influential factors in reducing *S. cerevisiae* in verjuice, with the best efficacy accomplished at low juice depths. For combined treatments, 1-mm layer conditions were discarded as they did not allow effectively controlling juice temperature increases. FDA pasteurization requirements were achieved in verjuice exposed to 50 pulses at 45 °C for 20 min [PLMH45–3] and to 18 pulses at 47 °C for 8.5 min [PLMH47–3] using a layer of 3 mm. As a result, 5.10 ± 0.24 log CFU/mL (17 J/cm^2) (Fig. 2b) and 5.06 ± 0.08 log CFU/mL (6.12 J/cm^2) reductions (Fig. 2c) were obtained, respectively. These results were not statistically different from each other ($p > 0.05$). Fig. 2b & 2c shows that the inactivation of *S. cerevisiae* at 45 and 47 °C was significantly lower when the treatments were applied on a 5 mm-deep juice layer ($p \leq 0.05$). The maximal yeasts reduction (4.36 ± 0.25 log CFU/mL) was attained at 47 °C and 6.12 J/cm^2 [PLMH47–5]. In contrast, significantly lower reductions, i.e. 2.88 and 2.51 log CFU/mL, were achieved after PL treatments at 43 °C for 3 [PLMH43–3] and 5 mm [PLMH43–5] verjuice depths, respectively (Fig. 2a). Synergism between PL and MH treatments at above 40 °C has already been reported on the inactivation of L. *innocua* in buffer solutions (Hilton et al., 2017) and *Botrytis cinerea* and *Monilia fructigena* in nutrient agar (Marquenie et al., 2003). Marquenie et al. (2003) reported that MH inactivation of *B. cinerea* required 10 min at 45 °C, whereas only 3 min was needed when applying MH at 45 °C with 2 min of PL treatment. Thus, the combined treatment significantly decreased the exposure time compared to the individual treatments. As far as we are concerned, no information is yet available regarding the inactivation of yeasts in fruit juices by using combined pulsed light and MH treatments. However, the combined effect of continuous UV-C light (lethal wavelength region of PL) and MH on the inactivation of *S. cerevisiae* in fruit juices has been well documented (García Carrillo et al., 2018; Gouma et al., 2015). *S. cerevisiae* was reduced by 5 log after the treatment of apple juice at UV dose of 2.90 J/mL (2.7 min) and 57.5 °C (Gouma et al., 2015). Similarly, 4.7 log reduction of yeast was achieved in carrot-orange juice blend exposed to UV-C dose of 10 kJ/m^2 (15 min) at 50 °C (García Carrillo et al., 2018).

In conclusion, in the current study the application of 18 pulses for 8.5 min (6.12 J/cm^2) to a juice layer of 3 mm at 47 °C (PLMH47–3) was selected as the best pasteurization conditions of verjuice, as the visual color of the juice minimally changed under these conditions.

3.2. Effect of pasteurization treatments on the quality properties of Verjuice during storage

Physicochemical, antioxidant activity and optical properties of untreated (U), combined PL and mild heat (PLMH47–3) pasteurized and thermally pasteurized (P) verjuice right after treatment application is summarized in Table 4. All verjuice samples had similar pH values ($p > 0.05$) (Table 4a). Likewise, Caminiti, Noci, Morgan, Cronin, and Lyng (2012) found no difference between the pH values of an orange-carrot juice blend after PL treatment (3.3 J/cm^2) and pasteurization at 72 °C for 26 s, respectively. The TSS and TA of PLMH47–3 pasteurized verjuice were slightly higher than those values for untreated and thermally pasteurized verjuice ($p \leq 0.05$) (Table 4a). This is consistent with the results published by Gerard and Roberts (2004), who reported an increase in the TSS of apple juice after heat treatment.

Treated verjuice samples (PLMH47–3 and P) were more turbid than the untreated one ($p \leq 0.05$). The increased turbidity of fruit juices has been linked to heat treatment (Gerard & Roberts, 2004; Kaya, Yildiz, & Unluturk, 2015; Rivas, Rodrigo, Martínez, Barbosa-Cánovas, & Rodrigo, 2006). Gerard and Roberts (2004) indicated that the increased cloudiness of juice could arise from the interactions of haze-active proteins and haze-active polyphenols, which increase suspended particles in the juice.

Absorption coefficient was statistically higher for PLMH-treated juice than for untreated and thermally pasteurized verjuice (U, P) depending on the juice cloudiness and color. Müller, Noack, Greiner, Stahl, and Posten (2014) detected an increase in the absorption coefficients of both apple and grape juices after UV-C and UV-B irradiation, which was consistent with the color change of the juices.

PLMH47–3-treated verjuice samples were not statistically different from untreated and thermally pasteurized ones regarding their antioxidant activity (Table 4a). Likewise, earlier studies did not find a significant alteration in the antioxidant capacity of orange-carrot juice blend after PL treatment (Caminiti et al., 2012) and pomegranate and grape juices after PL and heat treatments (Pala & Toklucu, 2011, 2013a).

According to Table 4b, PLMH47–3-pasteurized verjuice exhibited lower L*, and higher a* and b* values compared to untreated and thermally pasteurized juices, possibly because PLMH at 47 °C induced browning of the juice. Aguilar, Ibarz, Garvín, and Ibarz (2016) detected the browning of nectarine juice treated after UV-C irradiation at 45 °C.

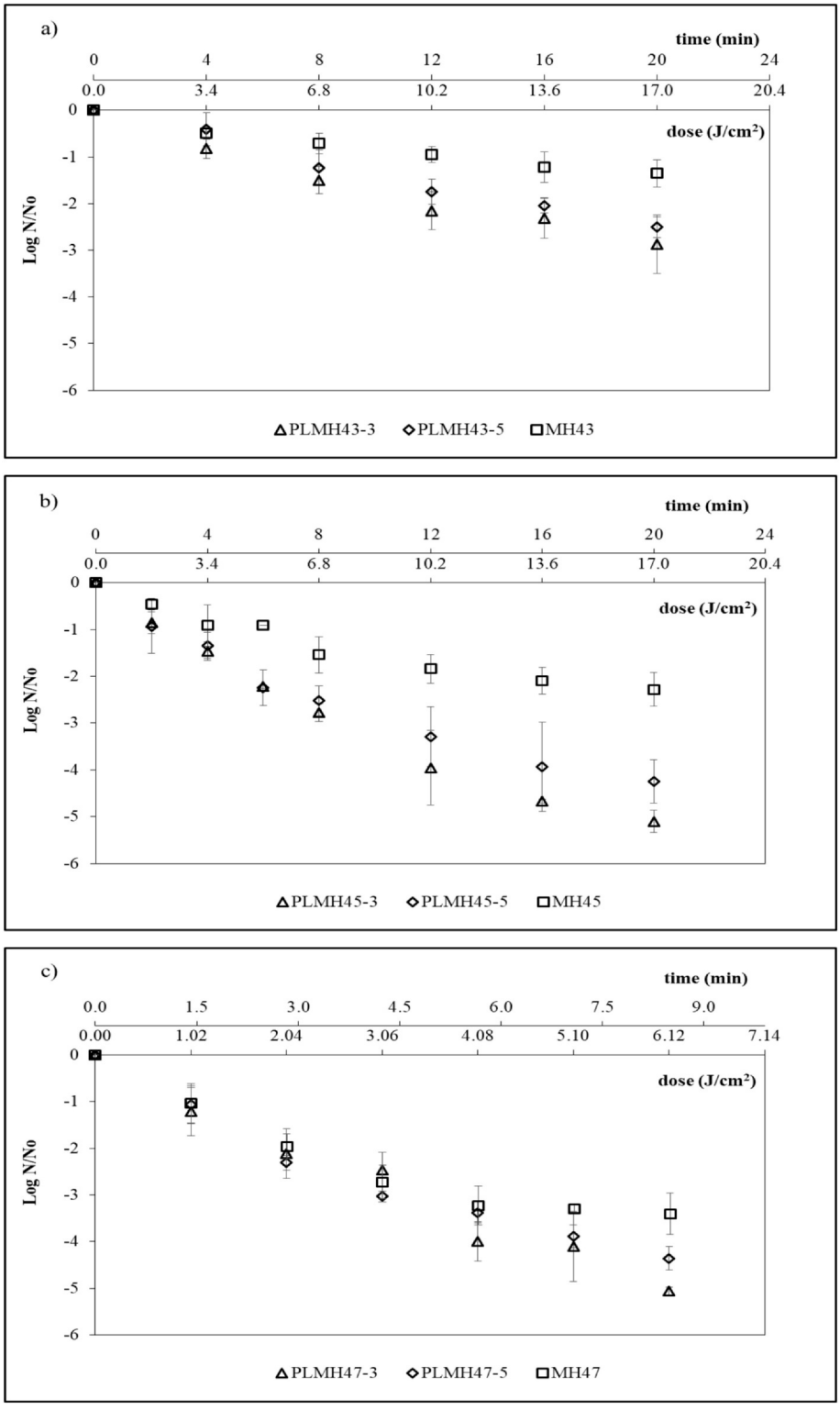

Fig. 2. The inactivation of acid-adapted *S.cerevisiae* in verjuice by MH and PLMH treatments at different temperatures a) 43 °C, b) 45 °C, c) 47 °C (□: Mild heating, ◊: Mild heating combined with PL at 5 mm juice depth, Δ: Mild heating combined with PL at 3 mm juice depth).

Bhat and Stamminger (2015) reported an increase of a* values could be due to the formation of melanoidin pigments in the juice. Also, Müller et al. (2014) determined the browning of apple and grape juices after UV-C irradiation, and stated that browning reactions in juice could occur as a result of the oxidation of phenolic compounds during the UV-C process. C* values suggest a slight color saturation after the combined PLMH47–3 pasteurization. A similar increase of saturation in UV-C irradiated pineapple juice was reported by Chia, Rosnah, Noranizan, and WD, W. (2012). Yellowness was similar among samples ($p > 0.05$). Considering CIE color parameters, the BI of PLMH47–3-pasteurized verjuice was slightly greater than other treated and untreated samples (Table 4b). Total color difference of PLMH47–3-pasteurized (ΔE: 1.54), and thermally pasteurized (ΔE: 0.69) verjuice were "slightly noticeable" according to the color classification given by

Table 4
Physicochemical, antioxidant activity and optical properties of untreated (U), combined (PLMH*) pasteurized and thermally pasteurized (P) verjuice. a) physicochemical and antioxidant activity properties, b) color properties.

a)

Treatment	pH		TSS (°Brix)		TA (%)		Turbidity (NTU)		Abs. coef. (cm^{-1})		AA (%)	
U	2.69	± 0.02a	4.20	± 0.00b	2.59	± 0.03b	158.0	± 1.73b	37.78	± 0.72b	91.50	± 0.35ab
PLMH*	2.67	± 0.01a	4.53	± 0.06a	2.83	± 0.02a	194.0	± 6.56a	41.61	± 0.83a	92.18	± 0.21a
P	2.67	± 0.01a	4.10	± 0.00c	2.59	± 0.03b	193.3	± 5.51a	36.62	± 0.58b	90.11	± 1.13b

b)

Treatment	L*		a*		b*		C*		h°		ΔE		BI	
U	24.91	± 0.36a	0.07	± 0.07b	4.16	± 0.11b	4.16	± 0.11b	88.96	± 1.01a	0.00	± 0.00a	17.86	± 0.62b
PLMH*	23.74	± 0.07b	0.28	± 0.05a	5.12	± 0.04a	5.12	± 0.04a	86.84	± 0.57a	1.54	± 0.36b	24.32	± 0.02a
P	24.42	± 0.23ab	0.08	± 0.04b	3.98	± 0.08b	3.98	± 0.08b	88.79	± 0.49a	0.69	± 0.22c	17.44	± 0.66b

Results were presented as "means ± standard error". The least significant difference was determined by Tukey pairwise comparison test. Means that do not share the same letter are significantly different ($p \leq 0.05$). *:PLMH47–3.

Cserhalmi, Sass-Kiss, Tóth-Markus, and Lechner (2006). Kwaw et al. (2018) also observed slightly noticeable color change after thermal and PL pasteurization of mulberry juice. They reported that these changes could result from the effect of heating and photochemical degradation reactions occurred in thermal and PL treatments.

The acceptable maximum total aerobic mesophilic bacteria count (TMAC), and yeast and mould count (YMC) in fruit juices are required to be 4 and 3 log CFU/mL, respectively (IFST, 1999; Turkish Food Codex, 2002). Based on this criteria, it was found that untreated verjuice were completely spoiled at 25 °C within 2 weeks (3.70 ± 0.80 log CFU/mL). However, no growth was detected in verjuice samples treated with PL assisted with mild heat (PLMH47–3) or in heat pasteurized during 6 weeks of storage at room temperature. Considering refrigerated verjuice samples, microbial growth was not observed in any sample throughout 6 weeks of storage. Ferrario and Guerrero (2016) reported an increase of 2 log in YMC of untreated apple juice after 6 days of storage at 5 °C whereas no change occurred in juices treated with a combination of PL and ultrasounds. Similarly, the number of *S. cerevisiae* increased by ~1.5 log in untreated apple juice after 7 days at 5 °C, but no growth was detected in PL-treated juice (71.6 J/cm^2) (Ferrario et al., 2015). Koh et al. (2016) were able to extend the shelf-life of cantaloupe fruit from 8 days to 28 days at 5 °C by applying repetitive PL (0.9 J/cm^2) to the fruit every 2 days. Various fruit juices could be extended by applying UV-C light under refrigerated conditions (Kaya et al., 2015; Pala & Toklucu, 2013b; Tran & Farid, 2004; Unluturk & Atilgan, 2015). Consequently, verjuice pasteurized with combined PLMH47–3 treatment was microbiologically safe after 6 weeks of storage under refrigerated (5 °C) and room temperature (25 °C) storage conditions.

pH, TSS, TA and antioxidant activity of the juice samples did not dramatically change over 6 weeks of storage at both temperatures. (Fig. 3a, b, c, f). TA was constant until week 4 and then began to increase slightly at week 6. Koh et al. (2016) documented no change in pH, TSS and TA of PL-treated cantaloupe during 28 days storage at 4 °C. Salinas-Roca, Soliva-Fortuny, Welti-Chanes, and Martín-Belloso (2016) found a slight change in pH and TSS of PL-treated fresh-cut mango during 14 days of storage at 4 °C. Elsewhere, these physicochemical values were generally unchanged during the shelf-life of fruit juices treated with PL and its combined technologies (La Cava & Sgroppo, 2015; Riganakos, Karabagias, Gertzou, & Stahl, 2017; Tandon, Worobo, Churey, & Padilla-Zakour, 2003).

Turbidity did not change during the first 2 weeks of storage at both temperatures. Then cloudiness of refrigerated juice samples significantly increased regardless the treatment applied until the end of the storage period ($p \leq 0.05$) (Fig. 3d). This might be related to the formation of tartrate crystals at cold conditions due to the inefficient detartarisation step (Andrés, Riera, & Alvarez, 1997). The increase in the cloudiness of untreated verjuice after 2 weeks at 25 °C could be explained by the growth of the molds within the juice (Fig. 3d). Furthermore, the increase in cloudiness of treated verjuice samples at 25 °C could be attributed to the formation of complexes between proteins and phenolic compounds in the juice composition (Lee, Yusof, Hamid, & Baharin, 2007). Additionally, the increment of turbidity in fruit juices could be explained by the inadequate inactivation of pectin methyl esterase enzyme, which is responsible for juice cloudiness (Rivas et al., 2006).

The absorption coefficient of verjuice samples stored at both temperatures exhibited different trends during storage (Fig. 3e). The absorption coefficient dramatically increased from 41.61 to 48.73 cm^{-1}, and from 36.62 to 44.47 cm^{-1} in the PLMH47–3-treated and P-treated juices, respectively over 6 weeks of storage at 25 °C. Guerrero-Beltrán and Barbosa-Cánovas (2004) indicated that the high cloudiness of fruit juice could result in a higher absorption coefficient and reduced light transmittance. The increase of absorbance in fruit juices during storage could result from the decomposition of colored compounds and the formation of dark-colored pigments (melanin and melanoidins) due to browning reactions (Müller et al., 2014; Unluturk & Atilgan, 2015). The absorption properties of melanin pigment and its effect on UV-C light irradiation was reported in literature (Seiji & Iwashita, 1965).

All untreated and pasteurized verjuice samples showed high antioxidant activity (AA) (Fig. 3f). The AA of untreated, PLMH47–3 and thermally pasteurized verjuice were 91.50, 92.18 and 90.11% at the beginning and did not markedly decrease at both storage temperatures. After 6 weeks, the AA of the untreated refrigerated juice was 91.06% while PLMH47–3-treated and thermally pasteurized verjuice exhibited 91.17 and 92.50% inhibition when refrigerated and 91.28 and 91.45% inhibition when stored at room temperature. Oms-Oliu, Odriozola-Serrano, and Martin-Belloso (2012) stated that UV-C light could change the AA of fresh plant products, depending on the dose, time and type of fruit. Kwaw et al. (2018) observed a slight decrease of AA of lactic acid fermented mulberry juice processed by PL assisted with and ultrasonic treatment, attributing to the degradation of phenolic contents. Additionally, the AA of the mulberry juice was better preserved in cold storage conditions. Koh et al. (2016) detected no change in the phenolic content of PL-treated cantaloupe during storage. Therefore, the unchanged AA may be associated to the stability of phenolic contents due to the high acidic conditions of verjuice (Kwaw, Tchabo, et al., 2018). Some studies report an increased AA in PL- or UV-C-treated fruit

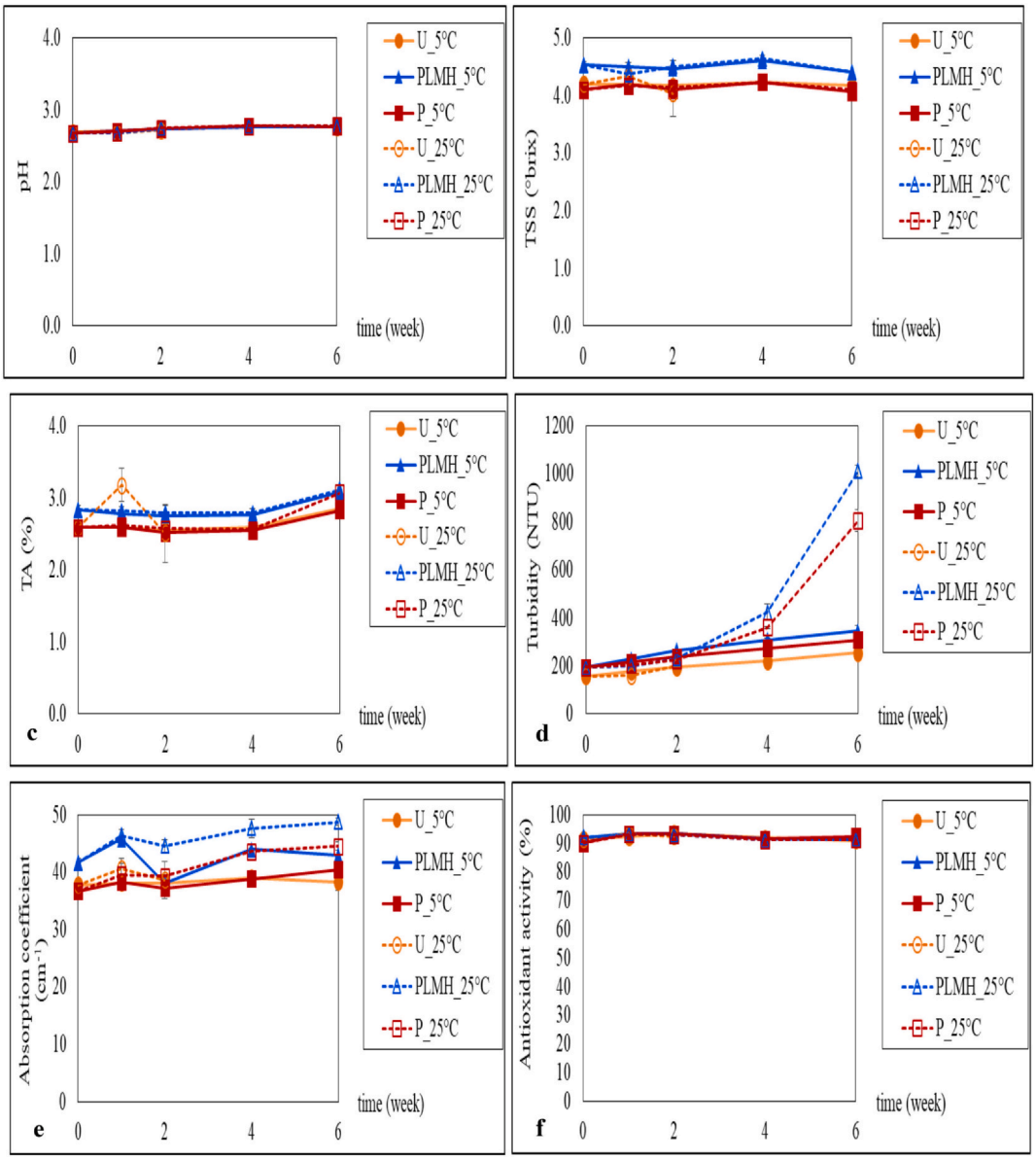

Fig. 3. Change in physicochemical (a, b, c, d, e) and antioxidant (f) properties of untreated (U: circle), treated with combination of processes (PLMH*: triangle) and thermally pasteurized (P: square) verjuice during 6 weeks of storage at 5 °C (solid line) and 25 °C (dotted line). *: PLMH47–3.

products during shelf-life by directly correlating their phenolic contents (González-Aguilar, Villegas-Ochoa, Martínez-Téllez, Gardea, & Ayala-Zavala, 2007; Oms-Oliu, Aguiló-Aguayo, Martín-Belloso, & Soliva-Fortuny, 2010; Pataro et al., 2015).

Lightness or L* value of all samples were almost the same through 6 weeks of refrigerated storage, whereas it slightly changed after 2 weeks at 25 °C (Fig. 4a). The most important variation occurred in a* values, all samples became significantly redder, particularly when stored at 25 °C. Under cold storage conditions, a* value of PLMH-treated juice increased by 2.84-fold, while it increased by 9-fold in untreated and by 7.5-fold in heat-treated verjuice (Fig. 4b). The increased a* value in verjuice could be due to the chlorophyll degradation or generation of brown melanoidin pigments during storage (Bhat & Stamminger, 2015). The combined treatment (PLMH47–3) maintained the green pigmentation in verjuice much better compared to the controls (U, P) under either storage conditions. Yellowness (b*) did not remarkably change at refrigerated temperature, whereas it markedly increased at 25 °C after the first week, and fluctuated until the end of storage period (Fig. 4c). The chroma (C*) change in all verjuice samples was much more noticeable at 25 °C (Fig. 4d). The hue angle of verjuice was around the yellow region (h°: 90°), which is in accordance to Pathare, Opara, and Al-Said (2013). It decreased by 8–9% in PLMH-treated verjuice after 6 weeks at both storage temperatures. However, it significantly decreased by 16% in the positive control (P) at 25 °C (Fig. 4e). No significant change in yellowness of the PLMH47–3-treated verjuice was observed. The total color change (ΔE) values for verjuice samples over 6 weeks of refrigerated storage were 1.24 (U), 0.79 (PLMH47–3) and 0.94 (P), which may be considered to be a slightly noticeable (0.5–1.5) change based on the classification given by Cserhalmi et al. (2006) (Fig. 4f). In contrast, the color change in verjuice samples stored at 25 °C was slightly higher and can be classified as noticeable (1.5–3.0). Browning index (BI) showed that color of verjuice samples was better maintained when stored under refrigerated conditions (Fig. 4g). By the end of the storage period, thermally pasteurized juice was 1.24-fold darker than the juice treated with PLMH47–3. These results are in accordance with literature. Ferrario and Guerrero (2016) reported no change in the L* and b* values of natural apple juice after PL treatment (0.73 J/cm^2) during 12 days of cold storage. Koh et al.

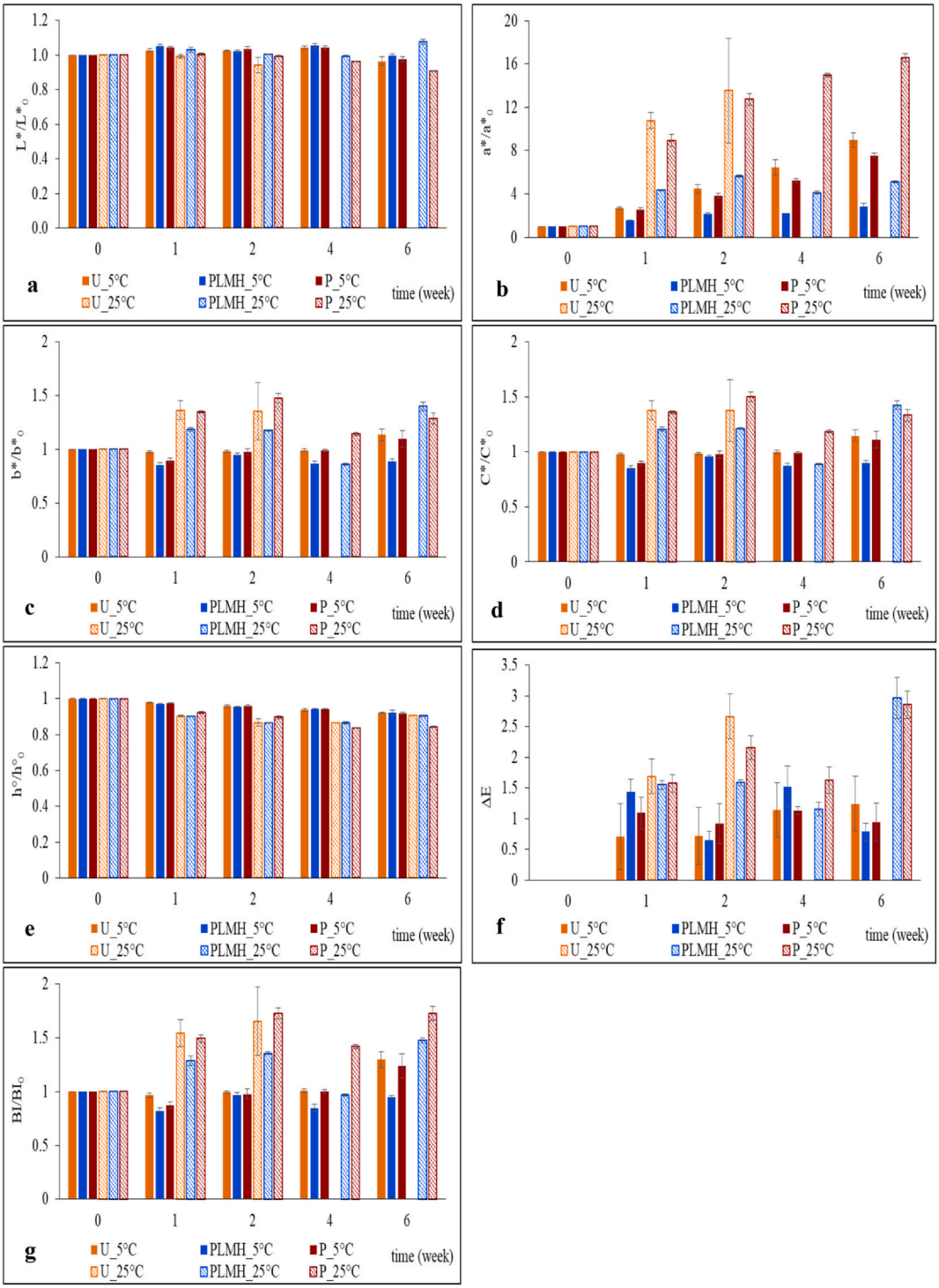

Fig. 4. Changes in color properties of untreated (U: circle), combined treated (PUVMH: triangle) and thermally (P: square) pasteurized verjuice during 6 weeks at storage of 5 °C (filled bar) and 25 °C (striped bar). a: L^*/L^*_0, b: a^*/a^*_0, c: b^*/b^*_0, d: C^*/C^*_0, e: h°/h°_0, f: ΔE, g: BI/BI_0. *:PLMH47–3.

(2016) did not observe any change in L*, C* and h° of repetitive PL-treated fresh-cut cantaloupe fruit during 28 days of storage at 4 °C. Salinas-Roca et al. (2016) also did not find a remarkable change in the L* and h° of PL-treated mango slices after 14 days at 4 °C. PL treatment has been reported to promote the formation of undesirable dark-colored melanoidin pigments through the interaction between phenolic compounds and oxidative enzymes (Ferrario & Guerrero, 2016). In this study, limited browning occurred in the PLMH47–3 pasteurized verjuice stored at refrigerated conditions, due to the restricted enzyme activity at cold storage and the highly acidic conditions of the juice.

4. Conclusions

Verjuice was successfully pasteurized by applying a combination of pulsed light and mild heat. Selected treatment conditions (6.12 J/cm^2 applied to a 3-mm juice layer. Together with heating to 47 °C for 8.5 min) allowed achieving 5-log reductions for *S. cerevisiae* and a significant shelf-life extension, up to 6 weeks, even under room

temperature storage conditions. Although the physicochemical properties of the pasteurized verjuice did not markedly change, the optical properties slightly changed during shelf-life.

In conclusion, the combination of PL and MH treatments allows meeting the FDA requirements for pasteurization of verjuice, thus extending the shelf-life while minimizing quality losses. This study reveals that PL technology can be an effective method for the pasteurization of acidic clear fruit juices, provided that an appropriate equipment can be designed by considering optimal process parameters.

Declaration of Competing Interest

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

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