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ORIGINAL PAPER

Pulsed Light Effect in Red Grape Quality and Fermentation

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Abstract *Vitis vinifera* (variety Tempranillo) grapes were processed by pulsed light (PL) at several treatment conditions before fermentation. The effect on grape external structure, wild microbial populations, phenol extraction and wine composition was monitored. PL treatments decreased significantly microbial loads in grapes. After treatment, the grapes were inoculated and fermented with *Saccharomyces cerevisiae* following fermentation during 2 weeks with skins and seeds. Temperature was set at 22 °C. PL treatments control wild microorganisms, especially yeasts, facilitating the growth and development of the yeast starter affecting wine quality. PL treatments of grapes can help to increase the shelf-life in postharvest technology but also improve fermentative purity and wine quality. PL is cheaper and easier to scale-up at winery than high hydrostatic pressure, pulsed electric fields or e-beam irradiation.

Keywords Pulsed light · Red grape · Yeasts · Cold pasteurization · Winemaking · Emerging technologies

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Abbreviations

PL	Pulsed light
HHP	High hydrostatic pressure
PEF	Pulsed electric fields
5fme	Treatment with five flashes at medium energy
10fme	Treatment with ten flashes at medium energy
5fME	Treatment with five flashes at maximum energy
10fME	Treatment with ten flashes at maximum energy

Introduction

Pulsed light (PL) is an emerging technology used in food preservation since late 1970s in Japan; its first patent was published in 1984 (Hiramoto 1984). High-intensity flash lamps are used to produce broad-spectrum light from 170 to 2600 nm including UV, visible and IR radiation. The base of this technology is the high intensity applied in a very short time pulse (1 μs–0.1 s) what produces very big power ≈35 MW (Rowan et al. 1999). Light intensity is 10^5 fold sunlight intensity at seaside level. It is common to use 1–20 pulses per second (Morata 2010). The energy density of a single pulse normally is lower than 50 J cm^{-2}. PL is a non-thermal technology of food preservation protecting sensory quality of processed foods (Oms-Oliu et al. 2010).

The effect of PL in microbial inactivation has been reviewed in several previous works (Gómez-López et al. 2007; Elmnasser et al. 2007; Oms-Oliu et al. 2010). The main bactericidal activity is produced by UV light component (Rowan et al. 1999; Gómez-López et al. 2007) especially 254 nm because of the DNA inactivation by production of pyrimidine dimers. Besides, cell wall disruption, membrane damage and effects on cytoplasm have been observed. For many food pathogens, 5–6 log cfu mL^{-1} count reductions have been described. In *Saccharomyces cerevisiae*, cell wall

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permeation and damage in nuclear membrane and vacuoles have been observed by transmission electronic microscopy when two to three pulses of 0.7 J cm^{-2} were used (Takeshita et al. 2003).

Currently, several non-thermal food technologies are being explored in wine making to reduce wild microorganisms in grapes protecting at the same time wine sensory quality. Among them, high hydrostatic pressure (HHP), pulsed electric fields (PEF) and irradiation (e-beam radiation) have shown promising properties decreasing strongly wild yeast and bacterial counts (Morata et al. 2015a; Puértolas et al. 2009; Morata et al. 2015b). Furthermore, these techniques let producers the use of new biotechnologies like fermentations with non-*Saccharomyces* yeasts (Bañuelos et al. 2016) and reduction of doses in allergenic additives like sulphur dioxide (Santos et al. 2013). Non-*Saccharomyces* yeasts are currently extensively used in enology to improve sensory quality in wines (Ciani et al. 2010; Gobbi et al. 2013; Loira et al. 2014, 2015; Kulkarni et al. 2015). PL is a cheap and affordable technique compared with HHP, e-beam irradiation or PEF. The investment cost is lower than 100 k€ (personal communication Claranor 2017) when a HHP device is about 1 M€ (Morata 2010). At the same time, PL is a robust technology with low and cheap maintenance. Moreover, it is a continuous technique that can be used in the preprocessing line after grape destemming or in the sorting table.

Continuous 254 nm UV-C light (8.4 kJ m^{-2}, 15 min, 27 °C) has been reported as able to produce the breakage on epidermal cells of skins in *Vitis labrusca* L. (Fava et al. 2011). These disruptions potentially promote the release of anthocyanin and tannins from skins. As the UV-C intensity is higher in PL, the extractive effect can help to facilitate fermentative extraction of grape phenolic compounds and other molecules with repercussion in wine quality. Similar maceration effects have been previously described in other emerging non-thermal technologies such as HHPs, PEF, e-beam irradiation and ultrasounds (Morata et al. 2015a; Delsart et al. 2014; Morata et al. 2015b).

The aim of this work is to evaluate the antimicrobial effect of PL in grape processing, potential effects on the extraction of phenolic compounds from skins and the repercussion of this technique in wine quality.

Materials and Methods

Treatment of Grapes by PL

Vitis vinifera L. grapes (variety Tempranillo) were manually destemmed, and 250 g were vacuum-packed in LDPE bags (Times®, Times S.A., Madrid, Spain). The polymer shows a high transparency to UV light and especially at 254 nm. PL treatments of five or ten flashes were applied at medium or maximum energy (treatment with five flashes at medium energy (5fme), treatment with ten flashes at medium energy (10fme), treatment with five flashes at maximum energy (5fME), treatment with ten flashes at maximum energy (10fME)). The maximum energy provided by each pulse is 600 J when working with both xenon lamps. Treatment area was 20 cm wide and 14 cm deep, resulting in an energy density of 1.8 J cm^{-2}. Medium-energy treatments were set at 300 J. A double xenon lamp SteriBeam SBS XeMatic-2L-A PL device was used (SteriBeam Systems, Kehl/am Rhein, Germany). The spectral light produced by the lamps had 30% of UV light corresponding to 12% UV-C, 10% UV-B and 8% UV-A. Samples inside plastic bags were double face irradiated by PL. Treatments were performed at room temperature. After treatment, the samples were manually pressed and then placed in sterilized 250-mL Erlenmeyer flasks for fermentation. All experiments were conducted in quadruplicate.

Fermentations

After PL treatments, the vacuum-packed grapes were examined in a laminar flow cabinet; the grapes were gently crushed and transferred to sterilized 250-mL Erlenmeyer flasks. Samples of 5 mL were taken to analyse wild microbial population. The remaining must was inoculated with a liquid culture of the corresponding yeast *S. cerevisiae* strain 7VA (Dept. of Food Science and Technology, UPM, Madrid, Spain). The population was 10^8 cfu mL^{-1}. After inoculation, the musts were fermented isothermally at 22 °C in the absence of SO_2. Controls in spontaneous fermentation with wild yeast population were also undertaken both in non-PL-irradiated grapes and in those treated at medium and maximum energies with five and ten flashes, respectively (5fme, 10fme, 5fME and 10fME). Samples were taken at day 0 and at the end of fermentation (day 15) in order to count yeast and bacteria populations. Also, at the end of fermentation, all the wines were analysed to measure anthocyanins, total phenols, organic acids, sugars, ethanol and volatile compounds.

Yeast Populations

Viable yeast cells were determined by plate counting. In the sequential fermentations, total yeasts (*non-Saccharomyces* plus *Saccharomyces*) were determined on glucose chloramphenicol agar (AGC) after 3 days at 25 °C. *Saccharomyces* counts were determined by plating at 37 °C for 2 days (at 37 °C, most of wine non-*Saccharomyces* are unable to form colonies). Non-*Saccharomyces* counts were performed in synthetic lysine agar media (Oxoid, Hampshire, UK). Bacterial counts were determined by plating in PCA supplemented with nystatin (50 mg/L) after sterilization and incubated during 48 h at 30 °C. All the media were from Pronadisa (Barcelona, Spain).

Determination of Anthocyanins

The following anthocyanins and pyranoanthocyanins were determined using an Agilent Technologies (Palo Alto, CA) series 1100 HPLC chromatograph equipped with a diode array detector and a quadrupole mass spectrometer with an electrospray interface: delphinidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, malvidin-3-*O*-glucoside-acetaldehyde adduct (vitisin B, Vit B), malvidin-3-*O*-glucoside-pyruvate adduct (vitisin A; Vit A), malvidin-3-*O*-(6″-acetylglucoside)-pyruvate adduct (acetylvitisin A; Vit A Ac), delphinidin-3-*O*-(6″-acetylglucoside), petunidin-3-*O*-(6″-acetylglucoside), peonidin-3-*O*-(6″-acetylglucoside), malvidin-3-*O*-(6″-acetylglucoside), peonidin-3-*O*-(6″-p-coumaroylglucoside), malvidin-3-*O*-(6″-p-coumaroylglucoside) and malvidin-3-*O*-glucoside-4-vinylphenol and malvidin-3-*O*-glucoside-4-vinylguaiacol. Gradients of solvents A (water/formic acid, 95:5 *v*/*v*) and B (methanol/ formic acid, 95:5 *v*/*v*) were used in a reverse-phase Poroshell 120 C18 column (Phenomenex, Torrance, CA, USA) (50 × 4.6 mm; particle size 2.7 μm) as follows: 0–2 min, 15% B (working flow 0.8 mL/min); 2–10 min, 15–50% B linear; 10–12 min, 50% B; 12–13 min, 50–15% B linear; and 13–15 min, re-equilibration. Detection was performed by scanning in the 400–600-nm range. Quantification was performed by comparison against an external standard at 525 nm and expressed as milligram per litre of malvidin-3-glucoside ($r^2 = 0.9999$). Anthocyanins were identified by their retention times and by comparing their UV–visible and mass spectra with data in the literature. Mass spectrometry was performed in positive scanning mode (*m*/*z* 100–1000, fragmentor voltage 150 V from 0 to 15 min). Twenty-microlitre sample of previously filtered (0.45 μm membrane) wines was injected into the HPLC apparatus. The detection limit was 0.1 mg/L.

Analysis of Volatile Compounds by GC-FID

Volatile compounds were determined using an Agilent Technologies 6850 gas chromatograph (Network GC System) equipped with an integrated flame ionization detector (GC-FID) (Abalos et al. 2011). A DB-624 column (60 m × 250 μm × 1.40 μm) was used. The following compounds were used as external standards for calibration ($r^2 > 0.999$): methanol, 1-propanol, 1-butanol, 2-butanol, isobutanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-phenylethyl acetate, 2-phenylethyl alcohol, diacetyl, ethyl acetate, isoamyl acetate, isobutyl acetate, ethyl butyrate, ethyl lactate and hexanol. 4-Methyl-2-pentanol was used as internal standard. All compounds were purchased from Fluka (Sigma-Aldrich Corp., Buchs SG, Switzerland). The injector temperature was 250 °C and the detector temperature 300 °C. The column temperature was 40 °C (5 min), rising linearly by 10 °C/min until 250 °C; this temperature was then held for 5 min. Hydrogen was used as carrier gas. The injection split ratio was 1:10, the in-column flow rate 2.2 L/min, and the detection limit 0.1 mg/L. One hundred microlitres of internal standard (500 mg/L) was added to 1-mL test samples and filtered through syringe membrane filters (pore size 0.45 μm) (Teknokroma, Barcelona, Spain). They were then placed in 2-mL glass vials sealed with a PTFE/silicon septum. One microlitre of this filtrate was injected into the GC apparatus.

Analysis of Acetic Acid and Residual Sugars

Acetic acid, lactic acid and malic acid were measured enzymatically using an Y15 enzymatic autoanalyser (Biosystems, Barcelona, Spain).

Infrared Spectroscopy

The equipment OenoFoss™ (FOSS Iberia, Barcelona, Spain) using Fourier transform infrared spectroscopy (FTIR) was used to identify and quantify major compounds such as residual sugars and total acidity. This technique also determines pH value.

Ethanol Quantification

Ethanol was analysed by liquid chromatography with refractive index detection (LC-RI) using a Waters e2695 apparatus (Milford, MA, USA) equipped with a 2414 Refractive Index Detector. Analyses were performed using a Phenosphere XDB C18 column (4.6 × 150 mm, 5-μm particle size) (Phenomenex, Torrance, CA, USA). The solvent was Milli-Q water (in isocratic mode) at 0.4 mL/min. The temperature was set at 30 °C both in the column and in the detector. Calibration was performed using an external ethanol standard (Panreac, Barcelona, Spain). Samples were injected after filtration through 0.45-μm cellulose methyl ester membrane filters (Teknokroma, Barcelona, Spain). The injection volume was 2 μL.

Colour Determination and TPI

The colour of the wines has been determined by the use of a UV–visible (UV–vis) spectrophotometer 8453 from Agilent Technologies™ (Palo Alto, CA, USA) with a photodiode array detector and the use of a 1-mm path length cuvette. The absorption at three different wavelengths (420, 520 and 620 nm) was used to compare colour intensity and hue in all trials after the fermentation was achieved. The results were also expressed as the percentage of yellow, red and blue fractions present in each finished wine, obtained from the different absorption wavelengths (Glories 1984a, b).

Total polyphenol index (TPI) was analysed spectrophotometrically recording the absorbance at 280 nm of 1% diluted wine in water in a 1-cm path length quartz cuvette.

Sensory Evaluation

The sensory analysis of the wines produced was assessed with a panel of ten experts in wine tasting. The panel was constituted by members of the Chemistry and Food Technology Department of the School of Agricultural, Food and Biosystems Engineering at Technical University of Madrid (Spain). Fourteen different attributes were rated with a five-point scale from low perception (1) to high perception (5) except for the hue that was rated from red young wines (1) to orange aged wines (5). The attributes evaluated were colour intensity, hue, aroma intensity, aroma quality, herbs, flowers, fruitiness, reduction, oxidation, body, astringency, bitterness, general acidity and overall impression.

Statistical Analysis

Means and standard deviations were calculated and differences examined using ANOVA and the least significant difference (LSD) test. All calculations were made using the PC Statgraphics v.5 software (Graphics Software Systems, Rockville, MD, USA). Significance was set at $p < 0.05$.

Results and Discussion

Wild Microbial Populations in Grapes of Both PL-Treated Grapes and Controls

It must be considered that the initial yeast population was very low, below log 2 cfu mL^{-1}. Normal yeast counts in grapes range from 2 to 4 log cfu mL^{-1} (Fleet 2003). Our grapes were at the lower average value. However, the effect of PL was statistically significant reaching 50% of reduction of the initial population with the treatments of 5fme and 10fME (Fig. 1a). Similar results of *S. cerevisiae* inactivation were achieved by Kaack and Lyager (2007), when treating carrot slices with four to eight pulses. The other two treatments show also reductions in average counts of 20–30% because of the variations in the population of the replicates without statistical significance. Also, it is important to note that PL contains UV–vis-IR radiation with low-penetration power affecting only 0.7–1 mm inside the berry (Aguirre et al. 2014) and the shadow areas are protected from the antimicrobial effect. At industrial level, the exposition to PL will be more effective when berries are transported in roll conveyors that move the grape and at the same time rotate them, producing a better exposition to the PL radiation in the full surface. The effectiveness of PL treatment will remain the same as when using plastic films for food

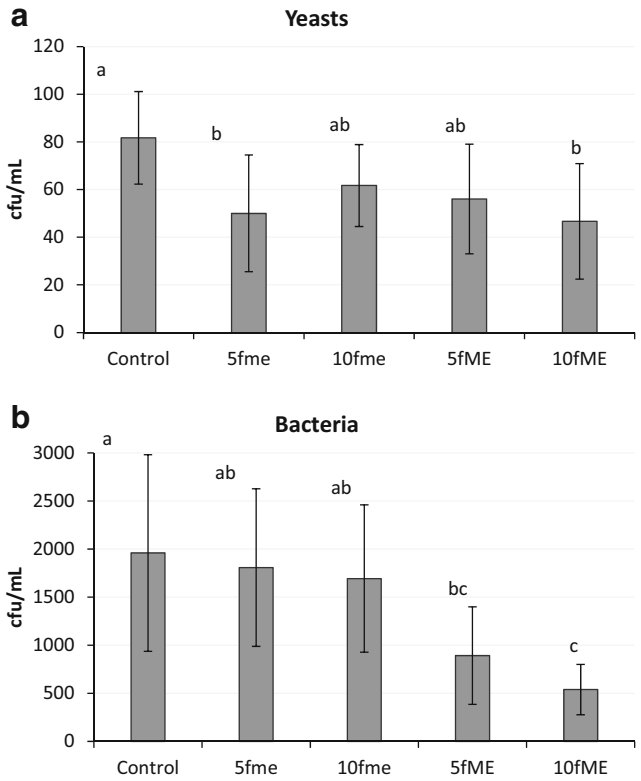

Fig. 1 Viable cell counts by plating in grapes after pulsed light treatments. **a** Yeasts and moulds. **b** Aerobic bacteria. Values are means ± standard deviations for four independent treatments. *Different letters* in the same series indicate significant differences between means ($p < 0.05$)

packaging (Fernández et al. 2009; Heinrich et al. 2015), since amorphous plastics are transparent in the range of wavelengths used in PL (Heinrich et al. 2015).

Similar antimicrobial results were observed when the specific media for non-*Saccharomyces* were used (data not shown), compared with total yeast populations. Therefore, the antimicrobial effect of PL in non-*Saccharomyces* is the same than in *S. cerevisiae*.

Bacterial counts were not affected by PL at medium energy at whatever number of pulses (5 or 10); however, when

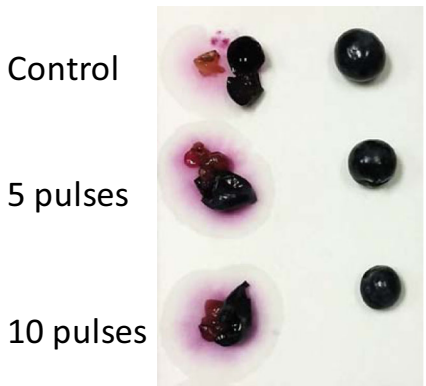

Fig. 2 Effect of pulsed light (five and ten pulses at total energy) in external appearance of grapes and in anthocyanin diffusion in pulp

Table 1 Enological parameters in wines analysed by enzymatic tests, IR spectrophotometry and LC-RID

Treatment	EtOH (% *v/v*)	Residual sugars (glucose + fructose) (g/L)	pH	Malic acid (g/L)	Total acidity (g/L)	Volatile acidity (g/L)	Lactic acid (g/L)
C	12.0 ± 0.2 a	1.5 ± 0.2 a	4.0 ± 0.0 a	2.7 ± 0.1 a	6.1 ± 0.2 a	0.8 ± 0.1 a	0.03 ± 0.03 a
5fme	11.3 ± 0.3 b	1.3 ± 0.1 a	3.9 ± 0.0 a	2.8 ± 0.1 a	6.2 ± 0.3 a	0.8 ± 0.2 a	0.02 ± 0.02 a
10fme	11.7 ± 0.4 ab	1.2 ± 0.2 a	4.0 ± 0.0 a	2.9 ± 0.2 a	6.0 ± 0.2 a	0.8 ± 0.1 a	0.03 ± 0.02 a
5fME	11.6 ± 0.6 ab	1.3 ± 0.1 a	3.9 ± 0.0 a	2.9 ± 0.2 a	6.2 ± 0.3 a	0.9 ± 0.1 a	0.03 ± 0.03 a
10fME	11.6 ± 0.5 ab	1.4 ± 0.2 a	3.9 ± 0.0 a	2.8 ± 0.1 a	6.1 ± 0.2 a	0.8 ± 0.1 a	0.03 ± 0.02 a

Values are means ± standard deviations of four independent fermentations. Different letters in the same series indicate significant differences between means ($p < 0.05$)

maximum energy is used, the counts were reduced to average 50% regarding the control ($p < 0.05$) when five pulses were used and to the 25% of the initial population when ten pulses were applied (Fig. 1b).

Effects on Grape Appearance and Structure

The external appearance of grapes remains unaffected compared with the control when PL treatments of five or ten pulses at total energy were applied (Fig. 2). This is in accordance with other authors who observed no skin damage or colour change in table grapes when treated with PL below 2 J cm^{-2} (Lagunas-Solar et al. 2006). After crushing the grape, the pulp is a little bit darker in grapes processed by PL than in controls. Maybe because of some cell disruption that facilitates anthocyanin diffusion inside the berry. This is in concordance with the microscopic breakage on skin epidermal cells observed by Fava et al. (2011). But this effect is not as strong as it was previously observed in pressurized (Morata et al. 2015a) or e-beam-irradiated grapes (Morata et al. 2015b).

Residual Sugars, Ethanol and Organic Acids After Fermentations in Grapes Processed by PL

All fermentations finished with residual sugar contents below 2 g L^{-1} and similar ethanol contents ranging between 11.3 and

Table 2 Volatile compounds in milligram per litre at the end of fermentation in control- and pulsed light-treated grapes

	Control	5fme	10fme	5fME	10fME
Methanol	84.6 ± 5.8 bc	81.7 ± 2.9 c	93.4 ± 2.7 ab	97.1 ± 2.6 a	95.2 ± 2.81 a
1-Propanol	46.4 ± 3.0 c	61.0 ± 3.1 b	64.4 ± 3.0 ab	72.9 ± 6.1 a	70.7 ± 1.10 ab
Diacetyl	3.8 ± 0.4 a	4.2 ± 0.4 a	3.7 ± 0.4 a	4.3 ± 0.2 a	3.9 ± 0.31 a
Ethyl acetate	33.3 ± 3.1 ab	32.3 ± 1.3 b	34.9 ± 4.0 ab	40.8 ± 4.9 a	39.7 ± 1.21 ab
2-Butanol	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.00 a
Isobutanol	26.1 ± 1.3 b	28.0 ± 1.0 ab	27.1 ± 1.6 ab	30.2 ± 1.7 a	29.1 ± 0.69 ab
1-Butanol	5.0 ± 0.4 a	5.3 ± 0.4 a	5.3 ± 0.5 a	5.7 ± 0.5 a	5.3 ± 0.55 a
2-Methyl-1-butanol	168.2 ± 8.6 c	173.2 ± 6.3 bc	172.7 ± 5.8 bc	190.7 ± 9.2 a	185.5 ± 4.98 ab
3-Methyl-1-butanol	40.1 ± 1.4 a	60.1 ± 6.9 a	44.9 ± 13.0 a	42.9 ± 5.4 a	58.0 ± 14.17 a
Isobutyl acetate	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.00 a
Ethyl butyrate	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.00 a
Ethyl lactate	14.0 ± 29.7 a	17.8 ± 29.7 a	11.1 ± 5.6 a	0.0 ± 0.0 a	9.4 ± 1.78 a
2–3 Butanediol	1329.3 ± 139.6 a	1426.6 ± 79.9 a	1246.9 ± 75.1 a	1472.6 ± 100.6 a	1347.4 ± 58.31 a
Isoamyl acetate	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.00 a
Hexanol	4.2 ± 0.1 a	4.5 ± 0.1 a	4.3 ± 0.1 a	4.5 ± 0.3 a	4.3 ± 0.11 a
2-Phenyl ethyl alcohol	63.4 ± 10.0 a	51.3 ± 4.5 ab	42.9 ± 4.0 b	42.6 ± 2.0 b	42.1 ± 2.57 b
2-Phenyl ethyl acetate	5.3 ± 0.1 a	5.2 ± 0.1 a	5.2 ± 0.1 a	5.2 ± 0.0 a	5.3 ± 0.07 a
Higher alcohols	364.1 ± 16.9 a	379.4 ± 6.7 a	360.3 ± 19.7 a	408.0 ± 36.2 a	387.1 ± 16.06 a
Esters	45.6 ± 10.3 a	40.5 ± 4.8 a	54.3 ± 4.6 a	48.2 ± 1.4 a	45.5 ± 0.85 a
Total volatiles	1992.5 ± 219.0 a	1936.7 ± 175.0 a	1907.7 ± 226.1 a	2020.7 ± 158.7 a	1995.7 ± 134.59 a

Values are means ± standard deviations of four independent fermentations. Different letters in the same series indicate significant differences between means ($p < 0.05$)

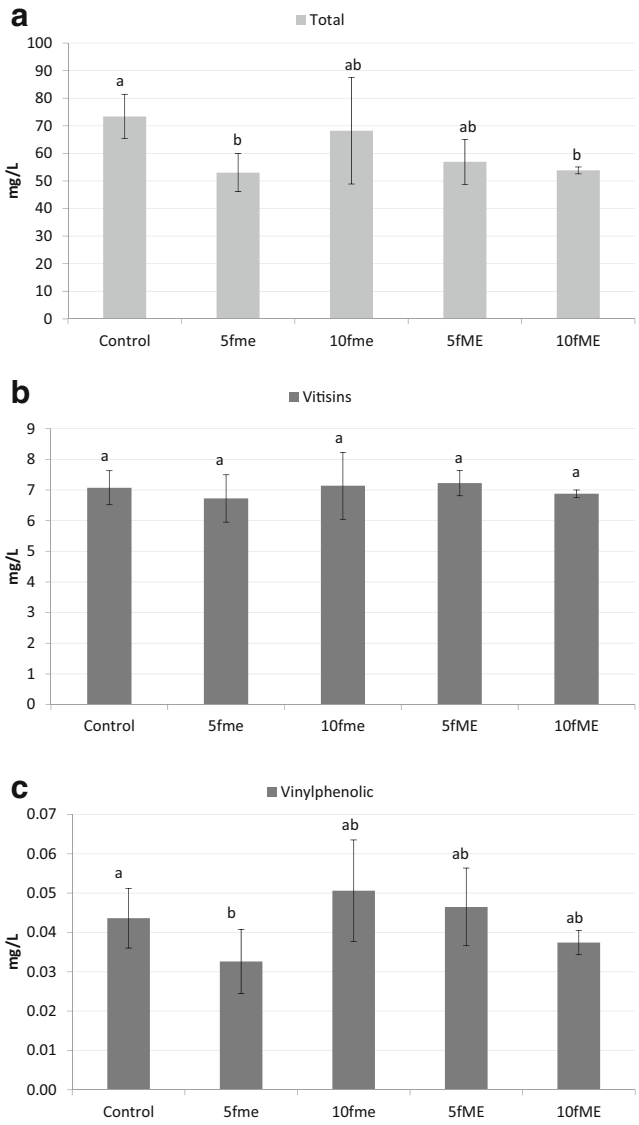

Fig. 3 Anthocyanin contents in mg L^{-1}: total anthocyanins (**a**), vitisins (**b**) and vinylphenolic pyranoanthocyanins (**c**), at the end of fermentation. Values are means ± standard deviations for four independent fermentations. *Different letters* in the *same bars* indicate significant differences between means ($p < 0.05$)

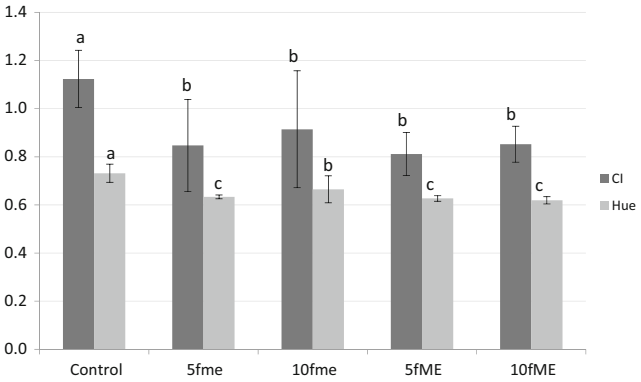

Fig. 4 Colour intensity and hue at the end of fermentation. Values are means ± standard deviations for four independent fermentations. *Different letters* in the *same bars* indicate significant differences between means ($p < 0.05$)

12.0% *v/v* (Table 1). The pH values did not show significant differences among treatments ($p < 0.05$), what means that the effect on the extraction by mechanical breakage because of PL energy did not affect the extraction of cations able to modify acidity/pH. Levels of organic acids were quite similar between treatments with levels of malic acid within 2.7–2.9 g L^{-1} and lactic acid lower than 0.03 g L^{-1} what means absence of malolactic fermentation by lactic acid bacteria. Concentrations of volatile acidity were in the range 0.8–0.9 g L^{-1}, higher than what is normal in *Saccharomyces* fermentations (Morata and Suárez-Lepe 2016), probably due to the experimental conditions used: small volumes and big headspace in Erlenmeyer flasks.

Volatile Fermentative Compounds

The enhancement of extraction in grape processed by PL can be observed by levels of methanol; this small alcohol is a constituent of the pectins that forms mainly the cell wall of grape skins supporting their structure and giving resistance. Normally, when the intensity of maceration processes increases during red winemaking, the levels of methanol also grow proportionally and also when enzymes are used to increase the extraction of phenols (Jackson 2014). In this case, the slightly higher levels of methanol in the PL-treated grapes regarding the control, especially in the stronger treatment, can be a consequence of the photothermic effect of the radiation in skin structure (Table 2). Similar effects have been observed in grapes processed by HHP (Morata et al. 2015a) and when grapes were processed at 10 kGy by e-beam irradiation (Morata et al. 2015b).

Regarding higher alcohols and esters, their formation has not been affected in a significant way by the use of PL in grape processing (Table 2). Thus, it indicates the possibility of PL irradiation being considered as a cold pasteurization of grapes, so that later emerging fermentation biotechnologies could be used with full guarantees of success, such as the use of non-*Saccharomyces* or selected *Saccharomyces* strains.

Anthocyanins and Colour

Even when the extraction is higher in PL-processed grapes and can be observed in the darker must colour just after crushing, the levels of anthocyanins at the end of fermentation are similar to controls or slightly lower in treatments 5fme and 10fME (Fig. 3a, $p < 0.05$). This can be produced by some photodegradative effect by PL oxidation; similar results have been previously observed in treatments with UV-C continuous light producing degradations between 8 and 16% of anthocyanins (Pala and Toklucu 2011). But frequently, this effect is lower than anthocyanin degradation in thermal processes. Oxidative degradation of pigments also has been observed

when e-beam irradiation is used in grape processing (Morata et al. 2015b). Stable pigments like vitisins show more stability without significant differences ($p < 0.05$) with control (Fig. 3b). This could be because of the higher stability of pyranoanthocyanin pigments (Bakker and Timberlake 1997), due to the higher number of resonant structures in the pyrano rings. A similar situation can be observed in vinylphenolic adducts also with a pyranoanthocyanin (Fig. 3c).

The lower level of anthocyanin pigments in wines from grapes processed by PL makes the colour intensity (CI) decrease by 10–20% (Fig. 4). However, a CI of 0.8–0.9 (range of PL-treated CI) instead of 1.1 (control CI) is not a big difference that could depreciate quality because in fact 0.8–0.9 is quite high. Maybe in wines or varieties with very low colour, this effect should be considered more carefully. Also, statistical differences can be found in hue values.

Sensory Analysis

The effect of PL processing is not too much significant in sensory profile because all the wines show similar global impression taste and aromatic quality (Fig. 5). However, the effect on herbaceous flavours was significant ($p < 0.05$), so maybe PL is able to degrade or oxidize some compounds responsible for green notes in wines. This is interesting because it can be helpful in grapes with not enough maturity. Also, a reduction in varietal smell when grapes have been treated with e-beam irradiation has been observed (Morata et al. 2015b). Moreover, some decrease in oxidative smell is observed in PL-treated grapes perhaps because of the degradation by PL of the molecules responsible for oxidative flavours. The decrease in CI observed when wine colour was analysed spectrophotometrically is also observed in the glasses, because PL-treated wine colour is lighter than in control wine (Fig. 5).

Conclusions

PL is a reasonably cheap technology that can be used in continuous flow during preprocessing of grape allowing reduction in microbial counts. Control of microorganisms in grapes favours the application of non-*Saccharomyces* yeasts in sequential cultures and mixed cultures of yeast–lactic acid bacteria to improve wine sensory quality or technological management of wine production. Moreover, reduction of wild microorganism in grapes is another way to reduce sulphite levels in wines. It is very important to guarantee the full exposition of all grape surfaces to PL, so at industrial scale, the shadow areas should be minimized in order to increase the antimicrobial effectivity. One easy and suitable possibility is the use of roller conveyor belts to change grape position during irradiation. Also, this technique can be useful as postharvest technology to increase the shelf-life of grapes and other berries.

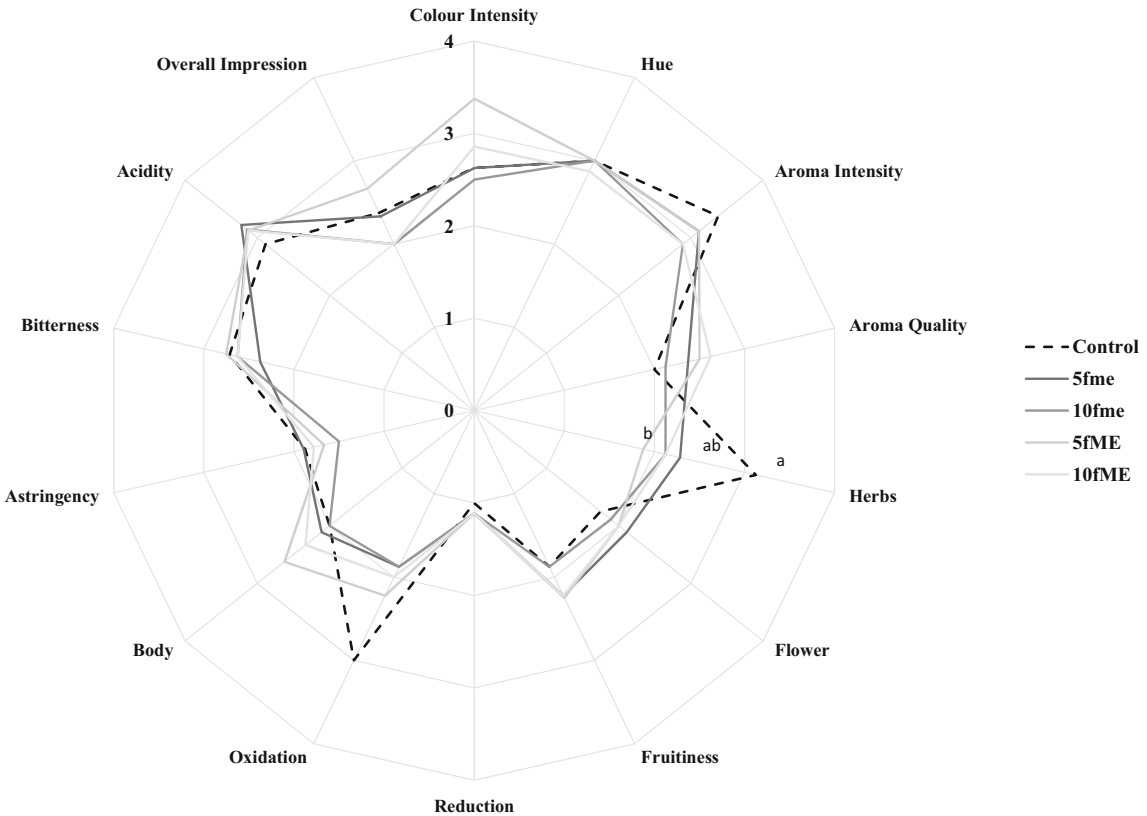

Fig. 5 Sensory evaluation. *Different letters* indicate significant differences between means ($p < 0.05$)

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