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(Article begins on next page)



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**EFFECTS OF CONTINUOUS EXPOSURE TO OZONE GAS AND ELECTROLYZED
WATER ON THE SKIN HARDNESS OF TABLE AND WINE GRAPE VARIETIES**

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ABSTRACT

The effects of continuous exposure to ozone gas (O_3 , 30 $\mu\text{L/L}$, 24 h) and immersion in electrolyzed water (EW, 400 mg/L free chlorine, 10 min) on the skin hardness of Italia and Muscat Hamburg table grapes and Merlot and Barbera wine grapes were investigated and compared with those of control treatments (air and deionized water, DW). Skin hardness was instrumentally evaluated via measurements of skin break energy using a puncture test. Ozone and electrolyzed water treatments generally increased skin hardness. For all of the cultivars and density classes, ozone treatment was more effective in increasing skin hardness than electrolyzed water treatment, although the differences were not always significant. For the same treatment, skin hardening was independent of berry density, and the magnitude of this effect was cultivar-dependent. This skin hardening might positively affect the postharvest life of table grapes. In wine grapes, increases in skin break energy values were related to higher yields and slow extraction kinetics of the phenolic compounds during maceration.

PRACTICAL APPLICATIONS

The purpose and the originality of this work were to determine the real impact of continuous exposure to ozone gas and immersion in electrolyzed water on skin hardness in table grape and wine grape varieties. Differences in the treatment effect due to the grape maturity level were studied in berries sorted according to density. For all cultivars and density classes evaluated, ozone treatment was more effective for increasing skin hardness than electrolyzed water treatment although the differences were not always significant. Therefore, the use of ozone gas as alternative sanitizing agent could extend the postharvest life of table grapes and facilitate the extraction of phenolic compounds from wine grapes during maceration according to increased skin hardness.

KEYWORDS: ozone gas; electrolyzed water; skin hardness; texture analysis; grapes

INTRODUCTION

Grapes used for fresh consumption, winemaking and raisin production are among the most cultivated fruits worldwide (FAO, 2013). Grapes are considered perishable fruits because their surfaces are characterized by the coexistence of various microorganisms, including filamentous fungi, yeasts and bacteria, that have different effects on the quality and shelf life of the final product (Barata *et al.* 2012). Table grapes are especially susceptible to fungal infection by *Botrytis cinerea*, which can induce gray mold disease and berry dehydration (Guentzel *et al.* 2010; Rolle *et al.* 2012a). In wine grapes, the growth of yeasts, such as *Brettanomyces* species, can result in off-flavors in wine that are primarily due to the formation of volatile phenols (Kheir *et al.* 2013).

Sulfur dioxide (SO₂) is an effective preservative that is commercially used to control the postharvest decay of table grapes and the microbial ecology of the must. However, its use is increasingly being restricted to avoid issues associated with sulfite residues, SO₂ emissions and berry damage that affects sensory quality (Feliziani *et al.* 2014). Currently, there is a considerable interest in research on alternative, safe and effective sanitizing agents such as ozone and electrolyzed water.

Ozone (O₃) is a powerful antimicrobial agent against a wide spectrum of microorganisms. The mechanism of microbial inactivation is complex because ozone attacks numerous cellular constituents (Khadre *et al.* 2001). In the gaseous and aqueous phases, ozone has previously been already as an environment-friendly alternative to traditional approaches for postharvest fruit and vegetable microbial control due to its high oxidizing power and rapid degradation that leaves not

residues (Horvitz and Cantalejo 2014; Karaca and Velioglu 2007; Sengun *et al.* 2014). Another alternative eco-friendly sanitizer is electrolyzed water (EW), which has been used to preserve the quality and safety of fresh-cut fruits and vegetables (Artés *et al.* 2009; Jemni *et al.* 2014). EW has a strong antimicrobial effect against pathogens and spoilage microorganisms due to the combined actions of hydrogen ions, its oxidation-reduction potential and free chlorine (Huang *et al.* 2008). In contrast to bleaching treatments involving hypochlorite, acidic and near-neutral EW contains primarily hypochlorous acid and thus leaves far less chlorine residue on the fruit (Guentzel *et al.* 2010). Therefore, human health and safety issues are minimized. Although the efficacies and sustainabilities of ozone and EW as antimicrobial agents for use in the control of the postharvest decay of grapes have been demonstrated, the effects of these treatments on the grape quality attributes have been minimally studied (Artés-Hernández *et al.* 2007; Botondi *et al.* 2015; Feliziani *et al.* 2014; Smilanick *et al.* 2002).

Texture is one of the most important quality characteristics of fresh fruits and plays a key role in the perceived quality and overall acceptability (Ha *et al.* 2007; Konopacka and Plocharski 2004). In table grapes, firmness and crunchiness are sensory quality traits that are highly appreciated by consumers (Sato *et al.* 2006). Instrumental measurements of the mechanical properties of the whole berry, flesh and skin have been used to objectively and quantitatively assess the textural quality of fresh table grapes (Giacosa *et al.* 2014; Río Segade *et al.* 2013a) and to monitor their postharvest shelf life (Deng *et al.* 2005). Specifically, the skin plays a key role in gas exchange regulation and berry resistance to injury and diseases during harvest and postharvest handling (Battista *et al.* 2015). In wine grapes, skin hardness permits an easy estimation of anthocyanin extraction kinetics with adequate reliability (Rolle *et al.* 2008).

There is no information available about the possible changes to the skin mechanical properties of grapes that are elicited during or after berry treatments with ozone or electrolyzed water. Therefore, the purpose of this study was to evaluate the effects of exposure to ozone gas and submersion in electrolyzed water on the skin hardness of table and wine grapes. The study was performed with *Vitis vinifera* L. cv. Italia and Muscat Hamburg table grapes and Merlot and Barbera wine grapes to assess the effects of the two treatments on fresh grape berries with different skin hardness. Furthermore, the table and wine grape varieties investigated in this study are highly appreciated and commercialized worldwide for fresh consumption and for the production of renowned red wines, respectively.

MATERIALS AND METHODS

Grape samples

The study was performed in 2014 with one white (Italia) and three red (Muscat Hamburg, Merlot and Barbera) *Vitis vinifera* L. cultivars grown in Italy. Italia and Muscat Hamburg table grapes were purchased from the local market. Barbera and Merlot wine grapes were harvested at an experimental vineyard located in the North-West Italy growing zone (Cuneo province). For each cultivar, approximately 10 kg of berries were randomly picked with short pedicels attached. To define the different maturity levels and to increase the intrasample homogeneity, the berries were sorted according to their density by flotation in saline solutions with sodium chloride concentrations ranging 100 to 190 g/L, which correspond to densities between 1069 and 1125 kg/m³ (Rolle *et al.* 2012b). The study was performed with berries belonging to the three most representative density classes, which accounted for total relative weights higher than 85% w/w as follows: A = 1069 kg/m³, B = 1075 kg/m³ and C = 1081 kg/m³ for Italia; B = 1075 kg/m³, C = 1081 kg/m³ and D = 1088 kg/m³ for Muscat Hamburg; D = 1088 kg/m³, E = 1094 kg/m³ and F = 1100 kg/m³ for Merlot; and G = 1107 kg/m³, H = 1115 kg/m³ and I = 1119 kg/m³ for Barbera.

The sorted berries were washed with water and visually inspected prior to treatment, and those with damaged skins were discarded.

For each cultivar and density class, two sets of 60 sorted berries were randomly selected for application to the ozone and EW treatments. The remaining two sets of 60 sorted berries were used as control samples; one group was exposed to the air, and the other was dipped in deionized water. Subsequently, the berries were placed into open polystyrene boxes in numbered positions. The remaining berries were subdivided into two replicates and used to determine the technological ripeness parameters of the grape juice obtained by manual crushing and centrifugation in a PK 131 centrifuge (ALC International, MI, Italy) for 5 min at 4000 g at 20 °C.

Technological ripeness parameters

Total soluble solid concentrations (°Brix, as SSC) were measured using an Atago 0–32 °Brix temperature compensating refractometer (Atago Corporation, Tokyo, Japan), the pH was determined by potentiometry using an InoLab 730 pH meter (WTW, Weilheim, Germany), and titratable acidity (g/L tartaric acid, as TA) was estimated using the OIV official method (OIV, 2008). Organic acids (citric acid, tartaric acid and malic acid) and reducing sugars (glucose and fructose) (g/L) were determined according to the methods of Giordano *et al.* (2009) using a 1260 Infinity HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector (DAD) set to 210 nm and a refractive index detector, respectively.

Ozone gas, electrolyzed water production and berry treatment

Ozone gas was produced using an ozone generator (Model C32-AG, Industrie De Nora SpA, MI, Italy) that was fed by an oxygen concentrator and had a nominal production capacity of 32 g O₃/h. The ozone gas was released into a sealed chamber. The ozone concentration in the

chamber was adjusted to 30 $\mu\text{L/L}$ and continuously monitored by the recirculation of ozone-enriched air from the chamber through a BMT 964 UV-photometric ozone analyzer (BMT Messtechnik GmbH, DE) that controlled the ozone generator output. The environmental conditions of the chamber were continuously recorded during the berry treatments using temperature and relative humidity data loggers (HOBO H8 RH/Temp, Onset Computer Corporation, Bourne, MA, USA). The sample boxes were introduced into the chamber, and the berries were exposed to ozone for 24 hours at a relative humidity of $57 \pm 3\%$ and a temperature of $20 \pm 1^\circ\text{C}$. Air exposed berries subjected to the same environmental conditions were used as control samples.

EW was produced from diluted salt (KCl) in tap water using an Eva System® 100 (Industrie De Nora SpA). The system produced EW of approximately 4 g/L free chlorine, pH 9 and 1% residual KCl. For the berry treatment, the original EW was diluted to 400 mg/L free chlorine using deionized water. Free chlorine concentration and pH were confirmed by iodometric titration (APHA, 1992) and potentiometry with an InoLab 730 pHmeter, respectively. Each berry was singularly immersed into 10 mL of EW for 10 minutes. The control samples were identically treated using deionized water (DW) rather than electrolyzed water (EW).

Berry skin mechanical parameters

A Universal Testing Machine (UTM) TA.XTplus texture analyzer (Stable Micro Systems, Godalming, Surrey, UK) equipped with an HDP/90 platform and a 5-kg load cell was used for the berry skin texture analyses. Skin hardness was evaluated with a puncture test using an SMS P/2N needle probe (Stable Micro Systems), a test speed of 1 mm/s and a penetration depth of 3 mm (Letaief *et al.* 2008). Each berry (before and after treatment) was individually punctured in the lateral face, and the skin break energy (mJ, as W_{sk}) was measured. The use of a needle probe

allowed for the independent estimation of skin hardness by minimizing possible interference from the pulp firmness. All data were acquired at 500 points per second, and the skin mechanical properties were calculated from force-distance curves using the Texture Exponent software package (Stable Micro Systems).

The berries were singularly evaluated before and 4 hours after the treatment. To allow for the estimation of the storage effects after treatment, the table grapes samples were also analyzed 7 days after treatment. Additionally, the wine grape berries that were treated with electrolyzed/deionized water were also analyzed 24 hours after treatment. This last evaluation was not considered appropriate for the ozone/air treatment samples of the wine grapes because they are typically processed shortly after harvest, and the postharvest treatment itself already accounted for 24 hours. The storage conditions were a relative humidity of $57 \pm 3\%$ and a temperature of 20 ± 1 °C.

The results were then expressed as the means of the differences between the berry skin break energy measurements after and before treatment, which were calculated individually for each berry and after-treatment measurement.

Statistical analyses

The statistical analyses were performed using the SPSS Statistics software package (IBM Corporation, Armonk, NY, USA). The Tukey-b test was used to establish significant differences at $p < 0.05$ following one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Technological ripeness parameters

The technological ripeness parameters of the Italia, Muscat Hamburg, Merlot and Barbera grapes sorted according to density are shown in Table 1. For each cultivar, the SSC values increased

significantly with increased berry density. The pH and TA values were not significantly different according to berry density, with the exception of the Merlot berries. For this cultivar, significant decreasing trends in the concentrations of tartaric, malic and citric acids were observed with increasing berry density, and these trends agreed with the TA values. In the table grapes, the SSC/TA ratios also increased significantly with increasing berry density. In the wine grapes, this ratio was not relevant. The changes in these chemical parameters with berry density agreed with those observed in previous studies of various table grape (Río Segade *et al.* 2013a,b ; Rolle *et al.* 2015) and wine grape (Rolle *et al.* 2012b; Zouid *et al.* 2013) cultivars.

Berry skin mechanical parameters

In this study, berry skin hardness was evaluated as skin break energy (W_{sk}). The Italia and Muscat Hamburg table grape and the Merlot and Barbera wine grape cultivars were selected according to the W_{sk} values of the untreated berries (0.578 ± 0.256 , 0.868 ± 0.292 , 1.041 ± 0.242 and 1.189 ± 0.350 mJ, respectively). These values were within the usual ranges for these cultivars given the variations maturity, climate, season, soil and viticulture (Giacosa *et al.* 2013; Río Segade *et al.* 2013b; Rolle *et al.* 2015).

A preliminary test that involved puncturing the berry skin in different equatorial places (5 punctures for each of 10 berries) was performed and revealed that for the studied cultivars, variations in W_{sk} (ΔW_{sk}) of approximately ± 0.08 mJ due to puncture position were observed. Letaief *et al.* (2008) previously reported that the effect of the berry puncture position (i.e., bottom, side and top) on the W_{sk} value is variety dependent. With the aim of considering only treatment effects and avoiding the variations inherent to the mechanical method, ΔW_{sk} values between -0.08 and +0.08 mJ were not taken into account.

Tables 2 and 3 illustrate the variations between the determinations of this mechanical parameter before and after the berry treatments in densimetrically sorted table and wine grapes,

respectively. For all cultivars and treatments, no significant differences were observed in the variations in skin hardness among the berries of the three studied density classes with the exception of the Barbera wine grapes at 24 h after EW treatment. Previously published studies have shown that W_{sk} values are not influenced by berry density in table grape (Río Segade et al., 2013a,b) or wine grape (Rolle *et al.* 2012b) cultivars.

With a few exceptions following the EW and DW treatments, the ΔW_{sk} values were positive, which indicated increased skin hardness after each treatment. Furthermore, these increases were significantly greater at 7 days after all treatments of the Italia and Muscat Hamburg table grapes and at 24 h after treatments of the Barbera wine grapes with EW and DW compared to the measurements taken 4 h after the treatments. This skin hardening has occurred during the postharvest storage of the fruit without significant berry weight loss. Increases in skin hardness are also observed during the on-vine dehydration process of wine grapes under uncontrolled environmental conditions (Rolle *et al.* 2010), and increases in the skin resistance to puncturing during postharvest dehydration have even been reported at 20 °C and 45% RH (Muganu *et al.* 2011).

When the effects of the different examined berry treatments on skin hardness were compared among the table grape cultivars, the highest ΔW_{sk} values were observed in the ozone-treated berries of any density class at 4 h and 7 days after treatment. The differences in the ΔW_{sk} values were not significant between the berries that were exposed to ozone and air or between the berries that were treated with EW or DW (the berries that were exposed to air and treated with DW were used as the control samples as mentioned above in the materials and methods section). Nevertheless, regarding the Italia grapes belonging to density classes B and C, the increases in skin hardness were significantly greater among the ozone-treated berries than among the EW-

treated berries. The percentages of berries with significantly harder skins ($\Delta W_{sk} > 0.08$ mJ) varied between 35.6% and 73.3% (following EW treatment, the density classes B and C exhibited values of 35.6% and 36.7%, respectively, and following ozone treatment, these values were 70.0% and 59.3%, respectively) at 4 h after berry treatment, whereas these values ranged from 73.3% to 94.4% (following EW treatment, the density classes B and C exhibited values of 78.0% and 73.3%, respectively, and following ozone treatment, these values were 91.7% and 89.8%, respectively) after 7 days. For the Muscat Hamburg grapes, significant differences in the ΔW_{sk} values were also observed between berries that were treated with ozone and those that were treated with EW at 4 h after treatment in the density class D and at 7 days after treatment in the density classes C and D. In these cases, the proportions of berries with significantly harder skins varied between 30.0% and 70.0% (following EW treatment, the density class D exhibited a value of 35.6% for EW, and this value was 70.0% following ozone treatment) at 4 h after berry treatment, whereas these values ranged from 71.1% to 96.7% (following EW treatment, density classes C and D exhibited values of: 74.6% and 71.1%, respectively, and these values following ozone treatment were 88.3% and 96.7%, respectively) after 7 days.

Rodoni *et al.* (2010) reported decreased pectin solubilization, polyuronide depolymerization and pectin methyl esterase activity in ozone-treated fruits. Reduced fruit softening following exposure to ozone might be related to reduced disassembly of cell wall pectic polysaccharides. Furthermore, the cuticle may also be a determinant factor of changes in the mechanical properties of fruit (Lara *et al.* 2014). Probably, this protective barrier reduces water loss following ozone treatment and could therefore increase cell turgor compared with other treatments, which would also explain the greater required skin rupture energy (De Belie *et al.* 1999). From the practical perspective, ozone-treated table grapes can be more easily handled postharvest due to their

greater skin hardness, and they can exhibit longer shelf lives due to postharvest microbial control (Horvitz and Cantalejo 2014; Karaca and Velioglu 2007).

Regarding the wine grape cultivars, the ozone-treated berries of all of the density classes also exhibited the greater skin hardening at 4 h after treatment compared with the DW- and EW-treated berries, with the exception of density class D Merlot grapes. However, significantly higher ΔW_{sk} values were observed only in the ozone-treated density class E Merlot berries and the density class I Barbera berries. For all of the wine grape cultivars of all density classes, the differences in the ΔW_{sk} values were not significant between the berries that were treated with EW and DW after 4 or 24 h. The percentages of Merlot berries with significantly harder skins ($\Delta W_{sk} > 0.08$ mJ) varied between 35.0% and 58.3% (the density class E berries exhibited values of 35.0% and 40.7% for the DW and EW, respectively, and 58.3% for ozone) at 4 h after berry treatment, whereas these values ranged from 31.8% to 65.0% among the Barbera berries (the density class I berries exhibited values of 31.8% and 32.8% for the DW and EW, respectively, and 61.7% for ozone). After 24 h, the proportions of Merlot berries that experienced skin hardening after the DW and EW treatments varied between 40.7% and 48.3%, whereas these values were 53.4% to 71.7% for the Barbera berries. Among all of the studied cultivars, the changes in skin hardness during storage were lowest for the Merlot berries, and the ΔW_{sk} values typically corroborated this finding at 4 and 24 h after DW and EW treatment.

Regarding the Merlot and Barbera wine grapes, the increases in skin hardness during exposure to ozone might have resulted in greater advantages in terms of the extractability of phenolic compounds compared with the DW and EW treatments. Higher skin hardness values promotes greater yields and slow extraction kinetics of anthocyanins from the skin (Rolle *et al.* 2008). This latter aspect is particularly favorable for varieties with high proportions of disubstituted

anthocyanins. These compounds are diffused during maceration faster than trisubstituted anthocyanins, but the former compounds are more easily lost during winemaking (Neves *et al.* 2008). Increased skin hardness can also favor the extractability of other phenolic compounds, such as flavanols (Rolle *et al.* 2011). The improved extractions of skin anthocyanins and flavanols would likely result in wines with higher color intensity, smoother taste and lower astringency.

CONCLUSION

In the food industry, the growing demand to minimize human health and safety issues has promoted the study of ozone and electrolyzed water as alternative sanitizing agents. Berry treatments with ozone gas and electrolyzed water typically induce increases in skin hardness, but exposure to ozone promotes significantly greater increases than immersion in electrolyzed water for berries with certain density values. However, within the same treatments, skin hardening was independent of berry density, and the magnitude of this effect was cultivar-dependent. This study showed the potential of ozone gas to extend the shelf lives of table grapes and to potentially facilitate the extraction of anthocyanins and flavanols from wine grapes during maceration due to increased skin hardness. Nevertheless, further studies are necessary to thoroughly evaluate other quality aspects of the obtained grapes and wines, such as chemical compositions, chromatic characteristics and sensory properties.

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1 **TABLE 1.** TECHNOLOGICAL RIPENESS PARAMETERS OF ANALYZED GRAPE SAMPLES SORTED ACCORDING TO
 2 DENSITY.

3

Cultivar	Density class	SSC (°Brix)	pH	TA (g/L tartaric acid)	SSC/TA ratio	Glucose/Fructose ratio	Citric acid (g/L)	Tartaric acid (g/L)	Malic acid (g/L)
Italia	A	16.6 ± 0.1a	3.62 ± 0.01	3.21 ± 0.01	51.02 ± 0.01a	0.948 ± 0.001a	0.16 ± 0.01	6.04 ± 0.01b	1.75 ± 0.01
	B	18.1 ± 0.1b	3.61 ± 0.05	3.19 ± 0.26	56.49 ± 4.18a	0.985 ± 0.001c	0.20 ± 0.01	5.72 ± 0.14b	1.52 ± 0.10
	C	19.4 ± 0.1c	3.72 ± 0.03	2.70 ± 0.08	71.99 ± 2.86b	0.978 ± 0.001b	0.15 ± 0.01	5.25 ± 0.02a	1.55 ± 0.04
	Sign ^a	***	ns	ns	*	***	ns	**	ns
Muscat Hamburg	B	17.7 ± 0.1a	3.62 ± 0.07	4.09 ± 0.27	44.21 ± 3.01a	0.999 ± 0.009	0.33 ± 0.01	3.58 ± 0.10a	2.88 ± 0.09b
	C	18.8 ± 0.1b	3.59 ± 0.02	4.16 ± 0.16	46.46 ± 1.75a	0.996 ± 0.008	0.32 ± 0.03	3.84 ± 0.01a	2.59 ± 0.15b
	D	21.1 ± 0.1c	3.71 ± 0.08	3.92 ± 0.03	55.38 ± 0.26b	0.969 ± 0.005	0.29 ± 0.01	4.46 ± 0.15b	1.83 ± 0.04a
	Sign ^a	***	ns	ns	*	ns	ns	**	**
Merlot	D	20.2 ± 0.1a	3.44 ± 0.02	6.30 ± 0.11b	-	1.029 ± 0.001	0.18 ± 0.01b	5.91 ± 0.05b	2.47 ± 0.03b
	E	21.5 ± 0.2b	3.43 ± 0.04	6.09 ± 0.24ab	-	1.031 ± 0.006	0.17 ± 0.01ab	5.91 ± 0.05b	2.16 ± 0.15b
	F	23.5 ± 0.1c	3.47 ± 0.02	5.59 ± 0.05a	-	1.019 ± 0.002	0.11 ± 0.02a	5.59 ± 0.06a	1.73 ± 0.07a
	Sign ^a	***	ns	*	-	ns	*	*	*
Barbera	G	24.6 ± 0.1a	3.13 ± 0.02	9.49 ± 0.05	-	1.010 ± 0.004b	0.34 ± 0.01c	7.87 ± 0.49	4.09 ± 0.09c
	H	25.5 ± 0.1b	3.13 ± 0.04	9.13 ± 0.40	-	1.007 ± 0.001b	0.30 ± 0.01b	7.46 ± 0.54	3.64 ± 0.02b
	I	28.7 ± 0.1c	3.04 ± 0.01	9.71 ± 0.05	-	0.994 ± 0.001a	0.26 ± 0.01a	7.83 ± 0.01	3.30 ± 0.01a
	Sign ^a	***	ns	ns	-	*	**	ns	**

4 All data are expressed as average value ± standard deviation (n = 2). Different Latin letters within the same column indicate
 5 significant differences (^a) among the three density classes for each cultivar (Tukey-b test; $p < 0.05$). Sign^a: *, **, *** and ns indicate
 6 significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively. A = 1069 kg/m³; B = 1075 kg/m³; C = 1081 kg/m³; D = 1088
 7 kg/m³; E = 1094 kg/m³; F = 1100 kg/m³; G = 1107 kg/m³; H = 1115 kg/m³; I = 1119 kg/m³. SSC = total soluble solids concentration;
 8 TA = titratable acidity; SSC/TA = SSC expressed as g/L and TA expressed as g/L tartaric acid.

TABLE 2. SKIN HARDNESS OF ITALIA AND MUSCAT HAMBURG TABLE GRAPES SORTED ACCORDING TO DENSITY.

Cultivar	Treatment	Density class	ΔW_{sk} after 4 hours (mJ)	ΔW_{sk} after 7 days (mJ)	Sign ^c
Italia	EW	A	0.088 ± 0.375αβ	0.361 ± 0.306	**
		B	-0.007 ± 0.276α	0.278 ± 0.342α	***
		C	0.008 ± 0.271α	0.258 ± 0.272α	***
		Sign ^a	ns	ns	
	DW	A	-0.023 ± 0.288α	0.408 ± 0.380	***
		B	0.013 ± 0.272αβ	0.272 ± 0.300α	***
		C	0.066 ± 0.323αβ	0.298 ± 0.333αβ	***
		Sign ^a	ns	ns	
	O ₃	A	0.220 ± 0.303β	0.543 ± 0.318	***
		B	0.206 ± 0.282β	0.495 ± 0.281β	***
		C	0.153 ± 0.269β	0.494 ± 0.351β	***
		Sign ^a	ns	ns	
Air	A	0.110 ± 0.198αβ	0.511 ± 0.242	***	
	B	0.101 ± 0.328αβ	0.441 ± 0.324αβ	***	
	C	0.038 ± 0.313αβ	0.366 ± 0.315αβ	***	
	Sign ^a	ns	ns		
Sign ^b			*, ***, *	ns, ***, **	
Muscat Hamburg	EW	B	-0.039 ± 0.310	0.349 ± 0.390	***
		C	0.020 ± 0.309	0.273 ± 0.338α	***
		D	-0.051 ± 0.302α	0.280 ± 0.400α	***
		Sign ^a	ns	ns	
	DW	B	0.008 ± 0.354	0.331 ± 0.448	***
		C	0.060 ± 0.319	0.341 ± 0.415αβ	***
		D	-0.017 ± 0.281α	0.360 ± 0.370αβ	***
		Sign ^a	ns	ns	
	O ₃	B	0.076 ± 0.342	0.432 ± 0.438	***
		C	0.108 ± 0.285	0.454 ± 0.334β	***
		D	0.234 ± 0.415β	0.548 ± 0.316β	**
		Sign ^a	ns	ns	
Air	B	0.072 ± 0.352	0.401 ± 0.413	***	
	C	0.085 ± 0.324	0.361 ± 0.386αβ	***	
	D	0.105 ± 0.314αβ	0.353 ± 0.243αβ	**	
	Sign ^a	ns	ns		
Sign ^b			ns, ns, **	ns, *, **	

All data are expressed as average value ± standard deviation (n = 60). Sign^{a,b,c}: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively, (a) among density classes, (b) among treatments and (c) among times after treatment. Different Greek letters within the same column indicate significant differences (b) among treatments for each density class and cultivar (Tukey-b test; $p < 0.05$). A = 1069 kg/m³; B = 1075 kg/m³; C = 1081 kg/m³; D = 1088 kg/m³. EW = electrolyzed water; DW = deionized water; O₃ = ozone gas; ΔW_{sk} = variation between skin break energy determinations performed before and after berry treatment.

TABLE 3. SKIN HARDNESS OF MERLOT AND BARBERA WINE GRAPES SORTED ACCORDING TO DENSITY.

Cultivar	Treatment	Density class	ΔW_{sk} after 4 hours (mJ)	ΔW_{sk} after 24 hours (mJ)	Sign ^c
Merlot	EW	D	0.056 ± 0.314	0.068 ± 0.282	ns
		E	-0.018 ± 0.288 α	0.058 ± 0.327	ns
		F	0.043 ± 0.312	0.028 ± 0.357	ns
	Sign ^a		ns	ns	
	DW	D	0.072 ± 0.282	0.093 ± 0.306	ns
		E	-0.007 ± 0.316 α	0.108 ± 0.298	*
		F	0.060 ± 0.314	0.034 ± 0.310	ns
	Sign ^a		ns	ns	
	O ₃	D	0.054 ± 0.299	-	
		E	0.141 ± 0.328 β	-	
		F	0.108 ± 0.338	-	
	Sign ^a		ns		
Sign ^b		ns, *, ns	ns, ns, ns		
Barbera	EW	G	0.082 ± 0.385	0.231 ± 0.424ab	*
		H	0.046 ± 0.384	0.403 ± 0.456b	***
		I	-0.039 ± 0.282 α	0.111 ± 0.379a	*
	Sign ^a		ns	***	
	DW	G	0.075 ± 0.431	0.231 ± 0.334	*
		H	0.013 ± 0.297	0.200 ± 0.423	**
		I	-0.032 ± 0.410 α	0.222 ± 0.355	**
	Sign ^a		ns	ns	
	O ₃	G	0.234 ± 0.412	-	
		H	0.110 ± 0.432	-	
		I	0.249 ± 0.585 β	-	
	Sign ^a		ns		
Sign ^b		ns, ns, **	ns, ns, ns		

All data are expressed as average value ± standard deviation (n = 60). Sign^{a,b,c}: *, **, *** and ns indicate significance at $p < 0.05$, 0.01, 0.001 and not significant, respectively, (a) among density classes, (b) among treatments and (c) among times after treatment. Different Latin letters within the same column indicate significant differences (a) among density classes for each treatment and cultivar (Tukey-b test; $p < 0.05$). Different Greek letters within the same column indicate significant differences (b) among treatments for each density class and cultivar (Tukey-b test; $p < 0.05$). D = 1088 kg/m³; E = 1094 kg/m³; F = 1100 kg/m³; G = 1107 kg/m³; H = 1115 kg/m³; I = 1119 kg/m³. EW = electrolyzed water; DW = deionized water; O₃ = ozone gas; ΔW_{sk} = variation between skin break energy determinations performed before and after berry treatment.