POSTHARVEST ELICITORS AND METABOLIC CHANGES IN WINE GRAPE BERRIES•

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1. INTRODUCTION

There is challenge to produce wines with a different range of organoleptic characteristics and/or high concentration of compounds that are beneficial for human health. Besides field operations and vineyard management, one option to modulate grape berry composition is represented by postharvest practices and/or treatments. After detachment, fruits (including grape berries) are still alive and metabolically active, so they react to the imposed environmental conditions and the presence of elicitors. One of the postharvest treatments applied to the production of raisins and to wine grapes is berry dehydration used to make special wines (often called dessert wines). These are characterized, in general, by high sugar and/or alcohol content, particular taste and aroma, resulting from both the concentration process and metabolic changes occurring in the flesh and the skin (Costantini *et al*. 2006; Rizzini *et al*., 2009). The general metabolism and the composition of the berries could be partly modulated and the loss of produce due to decay reduced when the withering process is carried out under a strict control of relative humidity, temperature and ventilation (Bellincontro *et al*., 2004). This occurs in dehydration chambers or tunnels where treatments with specific gaseous elicitors, such as carbon dioxide and ethylene, can be performed. CO_2 is effective in altering fruit metabolism and levels of about 2-5 kPa are applied in the Controlled Atmosphere technique for long-term storage of apples, pears, kiwifruit. Due to its toxicity, higher concentrations of CO_2 can be applied only for short periods with the aim, in general, of reducing fungal decay as observed in table grapes (Sanchez-Ballesta *et al.*, 2006) and improving quality traits such as the removal of astringency in persimmons (Yamada *et al*., 2002). In table grapes, a modulation in the expression pattern of specific phenylpropanoid genes (phenylalanine ammonia-lyase, PAL, chalcone synthase, CHS; stilbene synthase, STS) and in the accumulation of different phenol compounds, in particular a reduction in total anthocyanin and in *trans*-resveratrol, have been observed following treatments with 20 kPa of CO_2 (Sanchez-Ballesta *et al*., 2007).

The gaseous hormone ethylene is recognized as responsible for many of the changes occurring at ripening in climacteric fruit and the use of the hormone and/or of specific inhibitors of its biosynthesis or action is widely diffused in postharvest science and

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technology. Although grapes are classified as non-climacteric, ethylene seems to play some role during berry development and ripening. Applications at véraison of 2-CEPA, an ethylene-releasing compound, induce specific metabolic changes including an increase of anthocyanins by up-regulating expression of genes involved in their biosynthesis (El-Kereamy *et al*., 2003). Recently, specific information has been provided concerning the effects of ethylene treatments in harvested wine grapes to be used for the production of dessert wines after partial berry dehydration. Changes in cell wall enzyme activities and increase in terpenols, alcohols and C6 compounds have been detected in postharvest ethylene-treated grapes (Bellincontro *et al*., 2006; Botondi *et al*., 2009). The effects of ethylene treatment at véraison have been studied by a large-scale transcriptomic analysis via microarrays (Chervin *et al*., 2008) and a preliminary transcriptomic approach has been carried out on red-skinned wine grapes (cv 'Raboso Piave') treated, immediately after harvest, with 500 ppm for 7 days (Bonghi *et al*., 2010). Results indicate that, of the 139 genes identified as up-regulated at the end of the treatment, several are putatively involved in aromatic compound and polyphenol metabolic processes. These findings point out that application of ethylene in harvested grape berries induces marked changes in metabolism because of an altered pattern of gene expression.

In this paper we report some of the results obtained in white-skinned cv 'Trebbiano toscano' and coloured-skinned cv 'Sangiovese' treated, after harvest, with high CO_2 and ethylene concentrations, respectively, with the aim of better elucidating metabolic changes induced by these elicitors in berry skin tissue. We also report preliminary data concerning aromatic traits of Sangiovese wine obtained from ethylene-treated and untreated berries.

2. MATERIALS AND METHODS

2.1. Plant material and treatments

'Trebbiano toscano' grapes were harvested at technological maturity stage (about 18 °Brix) and berries were separated from the clusters, selected on the basis of size and appearance and incubated in small Plexiglass boxes at 20 °C under a continuous flow (100 mL min^{-1}) of air (control) or gas mixture (21 kPa O_2, 49 kPa N_2, 30 kPa CO_2) for 3 days. At the end of the 3-day treatment period, boxes of both control and treated berries were flushed with an air flux to allow them to dehydrate for further 9 days at 20 °C. At harvest (T0), at day 3 (end of the treatment), day 9 (3+6) and 12 (3+9) berry skins were frozen in liquid nitrogen and stored at -80 °C.

'Sangiovese' grapes were harvested in correspondence of about 22.7 °Brix and berries selected based on the size and skin color. Treatment with 1,000 ppm of ethylene was performed for 36h in Plexiglas boxes at 20 °C. Control berries were kept under an air flux. At the end of the treatment berries were maintained for further 48h in air and skin samples were collected, frozen in liquid nitrogen and stored at -80 °C. Microvinifications were carried out at the end of the 36h incubation period in ethylene and air.

2.2. Chemical analyses

2.2.1. Polyphenol extraction and quantification

Skin samples (0.5 g) were ground with liquid nitrogen. The plant material was extracted with methanol/water solution (80:20, v/v). The liquid extract was separated by centrifugation. The final volume was reduced to 16 mL by rotavapor, and then filtered with 0.45 μm filters Minisart and stored at -80 °C. Total phenols, flavonoids, (+)-catechin, and (-)-epicatechin were determined and quantified as described in Becatti *et al*. (2010). Total anthocyanins were assessed using the method described by Ribéreau-Gayon *et al.* (2003).

2.2.2. Total glycosylated and free aroma compound extraction and analysis

Glycosylated aroma compound determination in Sangiovese wine samples was performed using the Aubert *et al*. (2003) and the Cabaroglu *et al*. (2003) methods modified as follows. An amount of 15 g of PVPP, 300 ml of wine sample and 600 ml of water were mixed for 20 minutes and then filtered under vacuum and loaded into Lichrolut RP-18 cartridges. Samples were subjected to an over-night enzymatic hydrolysis (140 mg ml^{-1} of AR200 enzyme). To recover free aroma compounds, 100 ml of wine, added with 10 μl nonanol (3,22 mg L^{-1}), were extracted using dichloromethane and centrifuged (20 min, 7,000 rpm, 4 °C). The organic phase was added with anidrum sulphate and after a filtration the extracted samples were subjected to a distillation at 45 °C. GC analysis of volatiles was performed using an AGILENT 6890 MSD 5973, equipped with a FID, a fused capillary column (DB-Wax, 60 m x 0,250 mm i.d., 0,25 μm film thickness) and a AOC-5000 Auto Injector SHIMADZU, using helium as a carrier.

3. RESULTS AND DISCUSSION

'Trebbiano' skins markedly reacted to the presence of high (30 kPa) CO_2 concentrations for 3 days after harvest in terms of phenol compound metabolism. Compared with the samples at harvest (T0), total polyphenols and flavonoids decreased from day 3 and throughout the experimental period in the skins of the control berries (fig. 1). At the end of the 3-day treatment, CO_2 affected the maintenance of total polyphenol and flavonoid concentrations. Compared with non-treated berries, CO_2 treatment increased the skin concentration of total phenols and flavonoids at day 3+9. Unlike the control samples, a marked increase in both (+)-catechin and (-)-epicatechin concentrations was detected at the end of the CO_2 treatment followed by a significant decrease thereafter (fig. 1).

The effects of the 1,000 ppm ethylene treatment were monitored in terms of polyphenol and anthocyanin concentrations in the skins of 'Sangiovese' berries. Total phenols showed a decrease in control grapes throughout the experimental period and this confirms previous results showing that during a postharvest dehydration process a reduction of polyphenols occurs (Versari *et al*., 2001; Bellincontro *et al.*, 2004). During the gaseous treatment (36h), the decrease of polyphenol slowed down but a marked reduction of these compounds was observed after transferring berries to air for 48h (fig. 2a).

A similar trend was detected in terms of anthocyanins concentration that, differently from control samples, did not show any decrease throughout the incubation period in treated berries (fig. 2b). This effect was particularly evident for malvidin-3-O-glucoside, the most important anthocyanin pigment in coloured-skinned berries, which, differently

from cyanidin, did not show significant changes of concentration throughout the C_2H_4 treatment period (tab. 1).

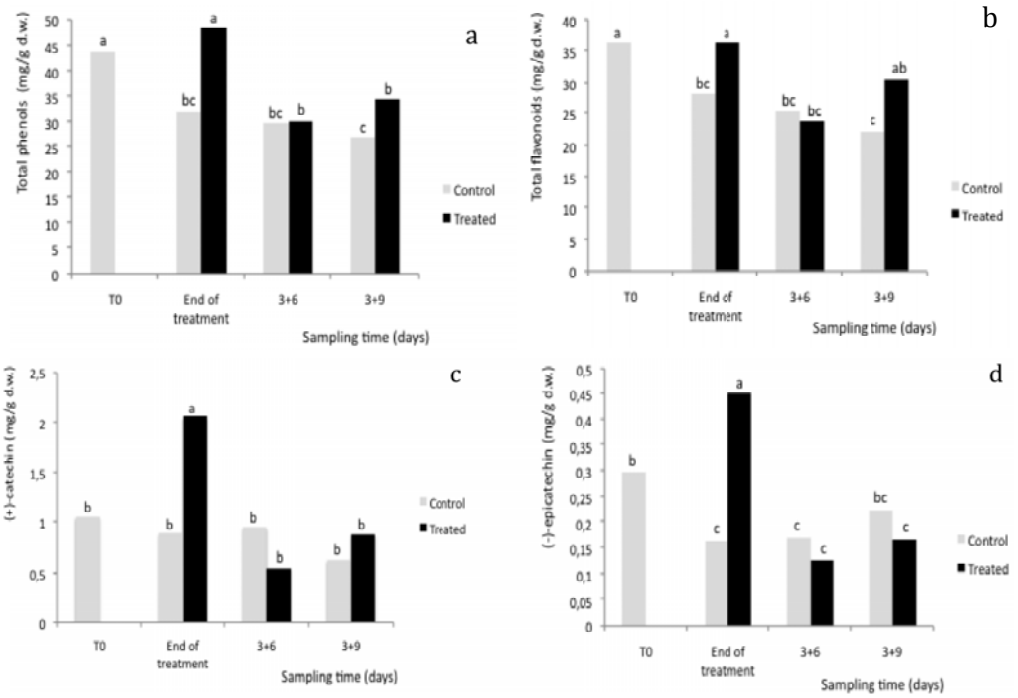

Fig. 1 - Total phenols (a), flavonoids (b), (+)-catechin (c), (-)-epicatechin (d) in the skin of 'Trebbiano' berries sampled at harvest (T0), at the end of the 3 day incubation period (end of treatment), and after 6 and 9 days of dehydration. Data represent the mean of three measurements. In this figure and in the following ones, for each sampling time different letters indicate statistically significant differences between means according to the Tukey-Kramer test (P=0,05).

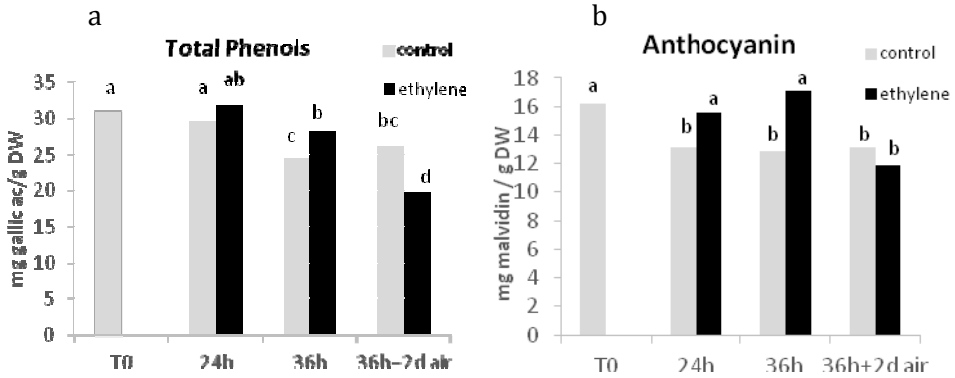

Fig. 2. Total phenol (a) and anthocyanin (b) content (mg g^{-1} dry weight) in 'Sangiovese' grape skin measured at harvest time (T0), after 24h (T1) and 36h (T2, end of the treatment) of incubation with ethylene, and 48h after the end of the treatment (T3). Control berries were kept in air. Data represent the mean of three measurements.

Our results confirm those of Bellincontro *et al.* (2006) that reported an effect of postharvest ethylene treatments in the maintenance of anthocyanin content in particular in the early stages of the dehydration process of 'Aleatico' berries. It remains to be elucidated whether this behaviour results from a *de-novo* synthesis, balancing the degradation occurring during the postharvest life or from an inhibition of degradation processes.

Tab. 1 - Single anthocyanin content (mg g^{-1} dry weight) in 'Sangiovese' grape skin determined at harvest time (T0), after 24h (T1) and 36h (T2, end of the treatment) of incubation with ethylene, and 48h after the end of the treatment (T3). In this table and in the following ones for each sampling time different letters indicate statistically significant differences between means according to the Tukey-Kramer test (P=0,05). Data represent the mean of three measurements.

	Malvidin-3-O-glucoside		**Cyanidine-3-O-glucoside**	
	Control	Ethylene 1,000 ppm 36 h	Control	Ethylene 1,000 ppm 36 h
T0	5,219 ± 0,556 a		2,399 ± 0,556 a	
T1	3.655 ± 0.188 b	4.612 ± 0.078 a	2.052 ± 0.188 a	2.011 ± 0.078 a
T2	3.446 ± 0.026 b	5.243 ± 0.076 a	1.8 ± 0.026 a	1.834 ± 0.076 a
T3	4.043 ± 0.592 b	4.048 ± 0.664 b	2.303 ± 0.592 a	1.076 ± 0.664 a

The wine produced from C_2H_4-treated berries resulted different in composition considering both glycosylated and free aroma compounds (tab. 2). A significant increase in both glycosylated C13 and terpenoids was present in the treated sample, although the most pronounced difference concerned the free aroma compounds. Indeed, free C13, phenols, and esters markedly increased, whereas total alcohols dramatically decreased in the treated sample. The reduced alcohol content could be the result of an altered metabolic activity of yeasts affected by the treatment. The increase of both phenols and carotenoid-derived C13 compounds might be due to the well-known effect of ethylene on these specific metabolic pathways in ripening fruit tissues. An altered pattern of volatiles was also observed by Bellincontro *et al*. (2006) and Botondi *et al*. (2009) that pointed out an enhancement of terpenols, esters, alcohol and C6 compounds induced by ethylene in 'Aleatico' berries. In this cultivar, similarly to what we found in 'Sangiovese', a higher total phenol content was detected in the wine obtained from ethylene treated grapes, whereas esters showed an opposite trend (Bellincontro *et al*., 2006).

Tab. 2 - Total free and glycosylated aroma compound concentrations (μg L^{-1}) measured in 'Sangiovese' wine samples obtained from ethylene-treated (1000 ppm for 36h) and untreated (control) berries. Data represent the mean of three measurements.

	Free		**Glycosylated**	
	Control	Ethylene 1,000 ppm 36 h	Control	Ethylene 1,000 ppm 36 h
Total C13	1807.5 ± 620.1 b	4196.7 ± 330.3 a	28.5 ± 7.5 b	52.9 ± 3.9 a
Total phenols	1030.2 ± 38 b	2858.4 ± 50.2 a	15.8 ± 4.5 a	25.8 ± 6.1 a
Total esters	11436.4 ± 5353.3 b	34850.5 ± 346.8 a	n.d.	n.d.
Total alcohols	223732.2 ± 9873.0 a	69587.5 ±16523.5 b	18.1 ± 1.3 a	16.2 ± 1.9 a
Total terpenoids	n.d.	n.d.	14.6 ± 6.0 b	26.6 ± 4.2 a

The change of the free/glycosilated aroma compound ratio observed in the wine obtained from treated grapes (tab. 2) suggests that glycosidases are activated/induced as observed by Botondi *et al*. (2009) in 'Aleatico' berries treated with 1,000 ppm ethylene.

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Abstract

Wine grape berries respond to postharvest treatments with specific gaseous elicitors in terms of metabolic changes and composition. Short-term (3 days) high (30 KPa) CO_2 treatment affects phenol compound concentration in skins of 'Trebbiano toscano' berries. In particular a transient increase of the (+)-catechin and (-)-epicatechin concentrations was observed in CO_2-treated samples. Even though grape berries are classified as non-climacteric, postharvest treatment with gaseous ethylene (1,000 ppm for 36h) on 'Sangiovese' berries was effective in maintaining total phenol and anthocyanin concentration. Ethylene treatment also affected the concentration of the wine aroma compounds (C13, esters, phenols) by increasing the free/glycosylated aroma compound ratio in the wine obtained from treated berries.

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