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Melatonin treatment of pre-veraison grape berries to increase size and synchronicity of berries and modify wine aroma components

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ABSTRACT

A comprehensive investigation was carried out to determine the effect of exogenous melatonin treatment of pre-veraison grapes on grape berries and its wines. Two melatonin treatments of pre-veraison grape berries increased the weight of the berries by approximately 6.6%. Meanwhile, this melatonin treatment could be beneficial in the reduction of underripe and overripe fruits and in enhancing the synchronicity of the berries. In addition, there were significant differences in the volatile compound composition between the wine produced from the melatonin-treated berries and the wines made from untreated berries. The wine from melatonin-treated pre-veraison grape berries had stronger fruity, spicy, and sweet sensory properties, compared to the wines made from untreated berries. Prolonging the treatment through repeated applications can enhance these effects and under different seasonal conditions, more pronounced effects on the grape quality and wine properties can be observed.

1. Introduction

The key determinant of wine quality is the composition of grape berries at the time of harvest. Berry composition at harvest depends on how and under what conditions each berry develops (Coombe & Iland, 2004). Therefore, grape quality is also referred to as wine quality potential. Berry ripening is an important stage during grape berry development for grape quality. Changes during this stage include berry softening; increases in sugar, anthocyanins, and aromatic compounds in the berry; malate decrease in the berry; accelerated growth of the berry; and colour change in the skin (Coombe, 1984; Coombe & Iland, 2004). Generally, berry ripening and grape quality are regulated by genetic, environmental, physiological, and chemical factors such as light, irrigation, temperature, and plant growth regulators. Plant growth regulators, including indoleacetic acid, gibberellin, ethylene, cytokinins, abscisic acid, and salicylic acid, are widely applied for grape cultivation.

Melatonin (*N*-acetyl-5-methoxytryptamine), a molecule with a low molecular weight and an indole-based structure, is ubiquitous in living organisms. Since the first report of melatonin in plants in 1995, melatonin has been identified in many different plants in a wide range of concentrations (from picograms to micrograms per gram of tissue) (Tan et al., 2012). Previous studies suggested that melatonin in plants is regulated by the transcriptional control of its biosynthesis genes (Kang, Lee, Park, Byeon, & Back, 2013; Park, Nguyen Le, Byeon, Kim, & Back, 2013). The presence of melatonin in grapes was reported in 2006 (Iriti, Rossoni, & Faoro, 2006) and was the result of an ongoing series of studies on the ability of agrochemicals to elicit the synthesis of phytoalexins in plants (Iriti, 2009). Phytoalexins are secondary metabolites with a broad spectrum of biological activities. The biosynthesis of phytoalexins represents an active plant defence response against biotic and abiotic stresses. However, the physiological and pathophysiological function of melatonin in plants is still unclear, although a hormone-like role has been putatively suggested in some plant species (Arnao & Hernández-Ruiz, 2007; Hernández-Ruiz, Cano, & Arnao, 2005; Iriti, 2009; Kolář & Macháčková, 2005).

The physiological functions of melatonin in animals are well recognised. However, little is known about its comprehensive effects in plants. Current evidence suggests that melatonin can protect plants against damage caused by various biotic and abiotic stresses (Tan et al., 2012). The physiological role of melatonin in plants might also involve regulation of their response to

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photoperiods (Arnao & Hernández-Ruiz, 2006), fruit development (Lei et al., 2013), delay of flowering (Kolář, Johnson, & Machackkova, 2003), and delayed leaf senescence (Wang et al., 2013). It also acts as a growth regulator, similar to indoleacetic acid (IAA), and may direct the differentiation of cells, tissues, and organs (Arnao & Hernández-Ruiz, 2006; Hernández-Ruiz, Cano, & Arnao, 2004; Hernández-Ruiz et al., 2005). Previous studies suggested that application of exogenous melatonin promoted lateral root regeneration and growth (Arnao & Hernández-Ruiz, 2007). Recently, an investigation conducted by Vitalini et al. (2011) identified melatonin in all grape berry tissues (skin, flesh, and seed). At pre-veraison, the highest melatonin content was found in the berry skin, whereas at veraison, the highest levels were in the seed. Furthermore, during ripening, melatonin decreased in the skin and increased in the seed and flesh. However, the role of melatonin during berry ripening it is still unclear. Chemically, melatonin is an indole derivative synthesised from tryptophan, which is similar to most auxins. Thus, it was speculated that melatonin might behave as an auxin in plant growth (Hernández-Ruiz et al., 2004). The effect of auxins on fruit development has been the subject of extensive studies and the outcome varies considerably depending on the auxin, plant species, and the developmental stage of the plant during application. A recent research on cherry fruits showed that a primary function of melatonin in cherry fruits is speculated to be as an antioxidant to protect the cherry from oxidative stress (Zhao et al., 2012).

In this study, we investigated the function of melatonin on berry ripening through spraying an exogenous melatonin solution on grape berries during pre-veraison. Our study demonstrates that exogenous melatonin has an effect on grape berry development, providing experimental evidence for further development and utilisation of melatonin as a plant growth regulator.

2. Materials and methods

2.1. Melatonin treatment of field-grown Merlot berries

The experimental vineyard was located at Yuquanying Farm (38.27°N, 106.06°E) of Ningxia Province, China. Fourteen-year-old Merlot (*Vitis vinifera* L.) grapevines, were grown on a flat terroir with well drained sandy soil in the eastern piedmont of the Helan Mountain. Natural rainfall was supplemented by limited drip irrigation as required. The vines were spaced 0.6 m within the rows and 3.0 m between the rows and the rows were oriented in a south–north direction. Vines were trained on a vertical single cordon positioning system with three wires. The vertical shoot-positioned canopies were uniformly managed and were trimmed twice manually, between bloom and veraison. A mixture of cymoxanil, mancozeb and carbendazim were used to prevent downy mildew, anthracnose, white rot, et al. There were no fungal or other diseases during the growing season. All uniform vines were divided into three groups. Bunches were sprayed once (July 5, 2012; MT-1) or twice (July 5, 2012 and July 15, 2012; MT-2) to run-off during the pre-veraison period, with 100 mg/L melatonin in 0.1% (v/v) Tween 80. Control fruit were sprayed with a 0.1% (v/v) solution of Tween 80. Veraison for the control fruit was determined to be on July 20, 2012. The trial was performed in triplicate with controls and melatonin treatments randomised over three adjacent rows. Each replicate consisted of fifteen treated vines (~400 bunches) with an untreated vine separating the different treatments.

2.2. Berry analysis

Berry weight was measured for each of the three replicates (100 berries per replicate). Individual Brix measurements were taken for all sampled berries ($n = 300$) using a TD-45 digital refractometer (Zhejiang Top Instrument Co., Ltd., Hangzhou, China). Melatonin content in different treated grape was detected with ELISA kits of Shanghai Jimian Bio-Technique Co. Ltd. (GS-E20467, Shanghai, China).

2.3. Small-scale wine making

Small-scale wine making was conducted in triplicate using the following protocol: control and melatonin-treated fruit (20 kg for each of the three replicates per treatment) were harvested at about 22 °Brix (September 15, 2012). The grape clusters were squeezed in a squeezing roller for de-stemming and crushing. After that, the grape must was transferred to stainless steel fermentation tanks (30 L) where 6% sulfurous acids were added to achieve 60 mg/L SO_2. Meanwhile, 30 mg/L pectinase (Lallzyme Ex, Lallemand, France) was added to the musts. After maceration of the musts for 24 h, 150 mg/L active dry wine yeast (*Saccharomyces cerevisiae* strain RC212, Lavlin, France) was added to the musts according to commercial specifications. The must was kept at 25 °C for about 10 days to allow for fermentation and maceration. Throughout this period, daily mass homogenisations were performed to dissolve the cap of the wine. Temperature and density were also recorded daily to evaluate fermentation arrests. Once fermentation and maceration were complete, the wine residue was removed from the vats and pressed. The marcs were discarded and the wine was recovered and decanted 15 days later, discarding the lees. Finally, the wine was bottled and aged for one month in the dark at 12–15 °C and each wine bottle was opened immediately prior to analysis. For each grape sample, three vinifications were performed.

2.4. Oenological parameters

The conventional oenological parameters including alcohol content, reducing sugar, pH value, total acidity, and volatile acidity of the wines at the moment of bottling were analysed according to the methods described by OIV (2014).

2.5. GC–MS chromatographic conditions

The aromatic components of the wine samples were analysed using stir-bar sorptive extraction followed by thermal desorption-gas chromatography–mass spectrometry (SBSE-TD-GC–MS) using a method validated by our group previously (Wen, 2013). Ten millilitres of wine sample were diluted with 10 mL of saturated salt water in a 20-mL vial, into which 20 μL of internal standard solution (2-octanol, 0.234 g/L) was added. A preconditioned stir bar (Twister) coated with polydimethylsiloxane (PDMS) phase (1 cm length, 0.5 mm thickness; Gerstel Inc., Baltimore, MD) was used to extract volatile compounds. The sample was extracted with the stir bar for 1 h at a speed of 1000 rpm. After extraction, The stir bar was then removed from the sample, rinsed with distilled water, dried with tissue paper, and later transferred into a thermal desorption tube.

TurboMatrix thermal desorption systems (TurboMatrix 350 Automated Thermal Desorber, PerkinElmer, Waltham, MA) were used to desorb the aromatic components from the stir bar. Helium was used as carrier gas and the desorption flow velocity was set at 45 mL/min. The heater valve and desorption tube temperatures were 245 and 270 °C respectively, with a 15-min desorption. Transfer line temperatures were set at 255 °C. Cold trap capture temperature was set at −30 °C with a rise to 255 °C set at 40 °C/min. The outlet split ratio was 3:1.

A Trace DSQ GC−MS equipment (Thermo-Finnigan, San Jose, CA) was used to analyse the aromatic components of the wine