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Control of environmental parameters in postharvest partial dehydration of wine grapes reduces water stress

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ABSTRACT

The hypothesis that a controlled environmental condition of postharvest partial dehydration of wine grapes may reduce berry water stress keeping a better grape quality, was verified on white wine grapes, var. 'Pecorino'. Grapes were harvested at 21.5% solid soluble content (SSC) and kept at 15 °C, 60% relative humidity (RH), and 2.5 m s^{-1} or 1.2 m s^{-1} air speed (tunnel), with a vapor pressure deficit (VPD) of 7.5–8 mbar; control bunches were kept in an uncontrolled condition (VPD = 0.7–6.4 mbar). Samplings for chemical and biochemical analyses were performed on grapes with 10, 25, 35, and 45% mass loss (m.l.). Tunnel and control samples reached 31.5% (in 24-26 days) and 28.5% (in 28 days) SSC, respectively. Control grapes had higher CO_2 production, alcohol dehydrogenase (ADH) and lipooxygenase (LOX) activities and proline content. Ethanol and acetaldehyde contents reflected ADH activity patterns. Control grape had higher percentage of branched alcohols than control ones. An activation of aerobic fermentation in control grapes is discussed with comment on related generation of specific volatile organic compounds (VOCs).

1. Introduction

The complexity of berry metabolism during partial dehydration has been recently shown by a survey of transcriptomic and metabolomic responses in berries, representing six grapevine genotypes subjected to postharvest dehydration under identical controlled conditions (Zenoni et al., 2016). A process of withering or dehydration or drying, which was considered as the only way to concentrate the berry juice to produce special wines, today it is recognized as a complex process where water stress and, depending on the technique, berry senescence occur. The same process of water loss from wine grape berries can be defined as drying, dehydration, withering, raisining (Mencarelli and Tonutti, 2013). But all these terms refer to special techniques or practices which, by using in different ways, the environmental condition surrounding grape bunches, produce different final products. In a microarray approach, Rizzini et al. (2009) demonstrated that the more pronounced the water loss of wine grape, the higher the number of differentially expressed genes while Bonghi et al. (2012) studied water loss from wine grapes, not only as the rate of water loss, but also as the intensity (amount) of the process; microarray data showed that e.g. two genes encoding for chalcone syntase were more down regulated in the slow process. The same Authors managed the environmental condition of dehydration to modulate the process speed because, changing temperature and relative humidity, VPD (vapour pressure deficit) is affected and it is the main driver of water loss. Also, temperature role in postharvest partial dehydration of wine grapes has been studied by Bellincontro et al. (2009) which found that carbon dioxide production rate from berries, partially dehydrated at 10 °C under higher air flow rate, was greater (~30%) than at lower ventilation, and similar to that of grapes partially dehydrated at 20 °C. Mencarelli et al. (2010) showed that relative gene expression of phenylalanine ammonia lyase (PAL), stilbene synthase (STS), chalcone isomerase (CHI), dihydroflavonol reductase (DFR) was upregulated in grapes partially dehydrated at 10 °C more than at 20 °C, at 20% m.l., while gene at was down regulated at 30 °C. Volatile compound profile changes with temperature during postharvest partial dehydration of grapes; the content of anaerobic metabolites such as ethanol, acetaldehyde, and ethylacetate was much higher at 20 °C than at 10 °C (Santonico et al., 2010). Franco et al. (2004), comparing wine from sun dried 'Pedro Ximenez' grape with wine from fully ripe grape found, in the former wine, higher concentration of acetoin, γ-butyrolactone, ethyl acetate, isoamyl alcohols, isobutanol, 2-phenylethanol, isobutanoic acid, and attributed this result to an anaerobic metabolism of 'Pedro Ximenez' grape berries during natural off-vine drying, Costantini et al. (2006) showed that two successive metabolic steps occurred if grape bunches were partially dehydrated under controlled environmental condition: the first, at 10–15% m.l., LOX was induced with formation of C6 aromatic compounds, the second, after 20% m.l., aerobic fermentation with higher

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ADH activity and alcohol production occurred. In this work, analyses of ABA (abscissic acid) and proline, were carried out showing an increase at slow intensity of dehydration, as it has been observed in vineyard during grape water stress (Deluc et al., 2009). In 2010, Toffali et al. found a similarity between the responses of ripe (to be considered a senescence process) and withered berries, concluding "However, the variation of metabolite profile during withering cannot solely depend on dehydration, because all molecules are not affected in the same manner". Zamboni et al. (2008) compared on-plant grape withering (overripening or senescence) and off-plant grape withering and found genes involved in hexose metabolism and transport, cell wall composition, and secondary metabolism (particularly the phenolic and terpene compound pathways) were similarly regulated in both processes.

The research activity whose result is reported in this paper, was stimulated by our hypothesis that a constant control of environmental parameters in postharvest partial dehydration of wine grapes, in comparison with a situation where environmental parameters were uncontrolled, might improve berry quality by permitting a greater resilience of berry cells. White wine grape cv. 'Pecorino' bunches were used and enzyme activities (ADH, LOX), protein and proline contents, and VOCs were analyzed during m.l. The effect of air flow rate on the berry biochemistry during dehydration was also monitored.

2. Materials and methods

2.1. Fruit material and experimental conditions

White wine grapes (*Vitis vinifera* L.) var. "Pecorino" grown in Abruzzo Region, Cataldi Madonna Winery, were carefully harvested at 21.5 ± 1.0% SSC and shipped to Postharvest Lab of DIBAF in Viterbo. Bunches with sound berries (no apparent trace of mould was observed), were dipped in a chlorinated solution (about 400 mg L^{-1} of sodium hypochlorite at pH 6.5) for few seconds, rinsed with tap water, and dried in front of fans. Bunches were then placed in perforated plastic boxes (60 × 40 × 15 cm) in a single layer to reach a weight of ~6 kg in each box. The plastic boxes (10 boxes each sample) were immediately stacked in two plastic tunnels built for grape partial dehydration (about 60 kg capacity), equipped with air speed controlled fans. The tunnels (controlled condition) were placed in a cold room to maintain the following environmental conditions: temperature, 15 °C (± 1 °C); RH, 60% (± 4%); air flow was set up to 2.5 (± 0.2) m s^{-1} or to 1.2 (± 0.2) m s^{-1}. For control, plastic boxes with bunches were placed in a room with open windows, monitoring temperature and relative humidity but without any control of them (uncontrolled); a fan was kept on to have air homogenization. The described set up of grape dehydration simulated what it is done traditionally. Postharvest partial dehydration treatment lasted the time to reach 45% (± 1%) m.l. in our bunches. Sampling was performed at the beginning and then at 10, 25, 35, and 45% m.l. During partial dehydration test, thermohygrometric conditions were monitored with a HYGROclip model probe (Rotronic AG, Bassersdorf, Switzerland) connected to HYGROwin software to record the data. Air speed was measured by means of a Terman hot-wire anemometer (LSI spa, Milan, Italy). 3 bunches of grape for each sample were placed in 1L glass jar (1 bunch in 1 jar) for 1 h, every day, to measure CO_2 concentration in the glass jar headspace, using an Helpy gas analyzer (Marvil Eng., Bolzano, Italy).

2.2. Chemical analyses

During partial dehydration process, three plastic boxes each sample were weighed, daily, to measure m.l. Thirthy berries were randomly picked from different bunches of each sample, squeezed separately and the juice was used for SSC measurement (model RL-2 table refractometer Abbe', calibrated at 20 °C, Officine Galileo, Florence, Italy).

Other berries were frozen in liquid nitrogen after seed removal, and stored at −75 °C for enzymatic assays of LOX and ADH, protein and proline determinations, and gas chromatographic (GC) analysis of VOCs. Proline determinations were carried out by applying the method of Bates et al. (1973). Protein content was measured using the BIORAD Protein Assay, which is a dye-binding test based on the Bradford assay (Bradford, 1976). Acetic acid was measured as volatile acidity using the method of L'Organisation International de la Vigne et du Vin (OIV) adapted to berry juice (OIV, 2014).

2.3. Enzymatic analysis

LOX was measured according to Bonnet and Crouzet (1977) with some modifications. All reagents, without specific reference, were from Sigma Aldrich Srl (Milan, Italy). Ten g of powdered frozen berries were suspended in 10 mL (1:1) of 0.5 M Tris-HCl buffer (pH 8.0) containing 1% w/v ascorbic acid, 1% w/v EDTA, and 1% w/v PVPP. The homogenate was centrifuged at 3500*g* for 15 min at 4 °C. A 1 M $CaCl_2$ solution (2% v/v) was added to supernatant and kept for 2 h to induce pectic substance precipitation. The mixture was centrifuged at 3500*g* for 20 min, and the supernatant was desalted using a PD-10 Sephadex G-25 M column (Pharmacia Biotech AB, Uppsala, Sweden) previously equilibrated with extraction buffer. All operations were carried out at 4 (± 1) °C. The assay mixture consisted of 100 µL of crude extract in 2.7 mL of 0.2 M buffer phosphate (0.2 M KH_2PO_4 + 0.2 M Na_2HPO_4*12 H_2O), pH 6.5 and 0.3 mL of incubation substrate prepared with 1 mL of 0.1 N NaOH, 5 µL of Tween 20, and 10 µL of linoleic acid; the final volume of 25 mL was reached adding bidistilled water. The reaction mixture was incubated at 37 °C in a water bath for 10 min. Enzyme activity was measured at 234 nm, with a Lambda 25 UV–vis spectrophotometer (Perkin-Elmer Instruments Ltd., Seer Green, Beaconsfield, U.K.), reading the rise in absorbance in 3 min, due to hydroperoxide formation. One unit of enzyme was defined as the change of 0.001 Abs at 234 nm for 1 min at 20 °C and expressed as µmol per g dry matter (DM). Dry matter instead of fresh weight was used to avoid the concentration effect in the computation, due to water loss during partial dehydration.

ADH was measured by applying Longhurst et al. method (1990), with some modifications. 5 g of powder obtained grinding frozen grape berries, were suspended in 10 mL (1:2) of 1 M Tris-HCl buffer (pH 7.4) containing 5 mM DTT, 1 mM EDTA, and 1% w/v PVP Polyclar-AT. The homogenate was centrifuged at 31,000*g* for 10 min at 4 °C and the supernatant used for the assay. The assay mixture consisted of 0.6 mL of crude extract in 2.7 mL of incubation substrate containing 0.1 M L-glycine (pH 9.6), 0.33 M NAD^+, and 0.2 mL of 0.3 M ethanol in water solution. Enzyme activity was measured at 20 °C by reading the rise in absorbance at 340 nm due to the NADH formation following the ethanol oxidation. Results were expressed as µmoles of NADH generated per minute per gram of DM.

2.4. Analysis of volatile organic compounds in grapes

VOC analysis of grape was performed by applying the SPME technique GC method described in our previous work (Costantini et al., 2006), slightly modified. Grape berry juice (5 g) was added to 5 mL of saturated $CaCl_2$ (1:1 w/v) and homogenized with 200 µL of standard solution (2-penten-3-one). The homogenate was collected in 25 mL glass miniflask and sealed with a Teflon silicone septum. The treated juice was exposed to a solid-phase microextraction fiber, respectively, for 30 and 15 min, in a Thermo Haake DL30-V15 B water bath (ENCO Spinea, Ve, Italy) maintained at a temperature of 20 ± 2 °C. The fiber, 100 µm PDMS (Supelco Inc., Bellefonte, PA) was conditioned in the GC injection port at 250 °C for 2 h before the use. After the selected extraction time, the SPME fiber was transferred to the injection port and thermally desorbed at 220 °C for 7 min. The splitless injector was mounted on a Trace GC, ThermoFinnigan UltraGC (ThermoFinnigan Inc., San Jose, CA) equipped with a fused silica capillary column impregnated with a polar phase of Carbowax 20 M (Alltech Associates