Postharvest Biology and Technology 137 (2018) 38–45

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Postharvest treatment with chitosan affects the antioxidant metabolism and quality of wine grape during partial dehydration

Milena Petriccione[a], Luca Pagano[b], Roberto Forniti[b], Luigi Zampella[a], Francesco Mastrobuoni[a], Marco Scortichini[a], Fabio Mencarelli[b,*]

[a] Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia agraria (CREA), Centro di ricerca per Olivicoltura, Frutticoltura e Agrumicoltura, Via Torrino 3, I-81100 Caserta, Italy

[b] Department for the Innovations of Biological, AgriFood and Forestry Systems, Postharvest Lab (LAPO) – University of Tuscia, Via De Lellis snc, 01100 Viterbo, Italy

ARTICLE INFO

Keywords:
Quality
Ozone
Antioxidant enzymes
Principal component analysis

ABSTRACT

The effectiveness of postharvest chitosan treatments on qualitative traits and antioxidant biochemical system during postharvest partial dehydration of "Sagrantino" grape has been studied, compared with ozone postharvest treatment. One % and 2% chitosan coatings delayed water loss but no difference in berry color or peel resistance during partial dehydration up to 30% mass loss (m. l.), was found among samples. The reducing sugar content rose straightly from 275 g L^{-1} (harvest), up to 445, 428, 411, and 390 g L^{-1}, in 2% chitosan, 1% chitosan, water, and ozone, respectively. Malic acid, and consequently total acidity, increased progressively in all samples with higher values in ozone- and in chitosan-treated berries. In all samples, total polyphenol content rose already at 10% m.l., and 1% chitosan sample had the highest value. Postharvest treatments enhanced the activity of antioxidant enzymes, superoxide dismutase (SOD) and ascorbate peroxidase (APX), during partial dehydration process, whereas inhibited polyphenoloxidase (PPO) and lipoxygenase (LOX) activity, preventing polyphenol loss and avoiding membrane oxidation, as shown by lower malondialdehyde (MDA) accumulation.

1. Introduction

Chitosan (poly-β(1–4)N-acetyl-D-glucosamine), a natural polysaccharide with a polycationic nature, known for its antifungal and eliciting properties, is considered an ideal coating to control decay of fresh fruit and vegetables due to its biocompatibility, biodegradability and bioactivity properties (Bautista-Baños et al., 2003; Bautista-Baños et al., 2006; Romanazzi et al., 2012, 2013; Shiekh et al., 2013; Xu et al., 2007; Wu et al., 2005). Just recently a book on chitosan and agricultural product preservation has been published where a numerous functional features of chitosan are reported in agriculture, food and environmental engineering (Bautista-Baños et al., 2016). Chitosan coating forms a semipermeable film that regulates gas exchange and reduces the transpiration rate, which is generally determined by the gradient of water vapor pressure between the fruit and the surrounding air (Bautista-Baños et al., 2006). Gao et al. (2013) showed that chitosan coating on table grapes reduced weight loss, and inhibited gas exchange and decreased nutrient loss; an induction of peroxidase and superoxide dismutase activities was also found. The effect of chitosan on weight loss by 1% chitosan postharvest treatment of table grape was confirmed by Al-Qurashi and Awad (2015) who measured a preservation of berry firmness, polyphenol content and antioxidant activity. Pre and postharvest treatment with chitosan on table grapes, controlled decay due to an induction of the activities of defense-related enzymes (polyphenoloxidase and phenylamoniolyase); in the same time, a decrease in soluble solid content (SSC)/acidity ratio and in weight loss was observed (Meng et al., 2008). Furthermore, chitosan postharvest treatment on table grapes to control *Botrytis cinerea* showed a positive effects on treated fruits with an increase in hydrogen peroxide, and in quercetin, myricetin, and resveratrol contents (Feliziani et al., 2013). In contrast, the same postharvest treatment on table grapes did not affect respiration and resveratrol content (Freitas et al., 2015) and these results were confirmed by Tessarin et al. (2016) in chitosan-treated wine grapes and derived wine. In conclusion, conflicting results on table grapes have been found and the reason is due to different form of application, different concentration, different application time. One thing seems to be confirmed: chitosan controls decay and decreases the weight loss.

Ozone has been tested for postharvest pest control in table grapes (Romanazzi et al., 2012; Feliziani et al., 2014) but, in the last few years, some publications have been done also on wine grapes (Carbone and Mencarelli, 2015; Botondi et al., 2015; Bellincontro et al., 2016;

* Corresponding author. Mencarelli Fabio Tel.: + +39 0761 357494
E-mail address: mencarel@unitus.it (F. Mencarelli).

https://doi.org/10.1016/j.postharvbio.2017.11.010
Received 8 September 2017; Received in revised form 10 November 2017; Accepted 10 November 2017

Laureano et al., 2016). Ozone is known as sanitizing agent but its effect as stressor has been also investigated (Heath, 2008). A significant increase in polyphenols in table and wine grapes has been observed (Artes-Hernandez et al., 2007; Carbone and Mencarelli, 2015; Bellincontro et al., 2016). Up to day it is unknown if this increase in grape polyphenols is due to a new synthesis or to an induction of antioxidant system. Yaseen et al. (2014) found on postharvest treated table grapes with ozone gas, a decrease in polyphenols, and an increase in catalase and no effect on lipoxygenase.

In Italy, the technique of partial dehydration is used for Amarone, one of the most worldwide famous dry wine. This technique induced a slow and long water stress on grape berry followed by a berry senescence (withering process). Just recently, an exhaustive survey in berries on six grapevine genotypes subjected to postharvest dehydration under identical controlled conditions has showed that this technique is not only a simple concentration process of the some substances due to water loss but a complex process that involved different transcriptomic and metabolomic responses (Zenoni et al., 2016).

If chitosan is effective in delaying water loss, then it could be useful for the withering process of wine grape berries during postharvest partial dehydration, and affecting positively the quality features of berry, e.g. increase of polyphenols. As the increase in polyphenols is a common result of chitosan or ozone treatment on grapes, then the study of the antioxidant biochemical system could be useful. In this paper results are reported on an experimental work on wine grape postharvest-treated with chitosan or ozone and then subjected to partial dehydration. Beyond important metabolic and quality features of wine grape, SOD, APX, PPO, LOX, and MDA were analysed.

2. Materials and methods

2.1. Experimental procedure and treatment

Grapes var. Sagrantino (*Vitis vinifera*) were harvested manually in the Terre della Custodia vineyard (Montefalco), sorting bunches with sound and turgid berries; SSC was 27 ± 1%. 123 kg of grapes were harvested and divided in four lots, about 30 kg each, consisting in: control (water), ozone, 1% chitosan, 2% chitosan.

Grape bunches, after berry sampling for chemical and physical initial analyses, were treated with chitosan (Iko Hydro, Rutigliano, Italy) with 90% deacetylation and a molecular weight of 360 kDa prepared at two different concentrations, 1% and 2% (w v^{-1}) in an aqueous solution of acetic acid (0.5% v v^{-1}). The solution was warmed to 45 °C and stirred on a magnetic stirrer for complete dissolution of chitosan, adjusting its pH to 5.2 with NaOH. After cooling at 15 °C, the fruits were dipped in the chitosan solution for 10 min and dried at room temperature.

Another lot of grape bunches was fumigated, overnight (10 h), with ozone (max 20 g h^{-1} with 6% w^{-1} of ozone) with a flow rate at maximum 150 NL h^{-1} (NL = normal litre) rate (Ozone generator A series, PC Engineering, Uggiate Trevano, Italy) in a 9 m^3 cold room, at 10 °C, relative humidity (RH) 80%. Control bunches (water) were immersed in water for 10 min as done for chitosan. After chitosan and control treatments, grape bunches were left to superficially dry in another cold room at 10 °C, RH at 80%, for 10 h, as for ozone treatment. Then, 6 perforated boxes, each one with 6 ± 0.5 kg of bunches for each treatment, were placed in small metallic tunnels (45 × 45 × 100 cm) fitted with an exhaust fan with airflow regulation (1.5 ± 0.3 m s^{-1}). The small tunnels were placed in a thermohygrometric controlled room at 20 ± 1 °C and 60% ± 5% RH for the partial dehydration treatment. The experiment lasted until grape bunches lost 40% of their mass but berry sampling for analyses was done initially, then at 10 (± 1), 18 (± 1), and 30 (± 1) % m.l. but not at 40%, because of the impossibility to extract juice from chitosan-treated berries.

2.2. Physical and chemical analyses

The mass of bunches (3 bunches per each box, total 18 bunches) was carefully measured using a technical balance (Adam Equipment Co.Ltd., Milton Keynes, U.K.). The color of 20 berries, sampled by cutting the berry with pedicel from different bunches (5 berries from 4 bunches of each treatment and sampling time), was assessed, at the indicated sampling times, with a CM-2600d colorimeter (Konica Minolta Inc., Ramsey, NY) set at SCE (specular component excluded), measuring CIELAB coordinates “L”, “a”, and “b”. After color reading, the same berries were analysed for peel resistance. Instron Universal Testing Machine mod. 3343 (Instron Ltd, High Wycombe, UK) was adapted with a 1 mm diameter flat probe and the bar speed was fixed to 10 mm min^{-1}. Berries were punched in the equatorial part, until the peel broke; two punches each berry. Data were expressed in terms of applied force (N), to break the peel resistance, to peel deformation (mm) until the time of peel break (N mm^{-1}). These berries were used to measure SSC by a digital refractometer (Atago CO. Ltd., Tokyo, Japan) and the values were expressed as%.

Juices from three set of berries of different bunches, each sampling time and each sample, were analyzed for reducing sugars, titratable acidity, malic acid, and total polyphenol content by following the OIV procedures (Organisation International de la Vigne et du Vin, 2009).

MDA content was evaluated following the modified method of Health and Packer (1968). Tissue powder was homogenized in ice bath by adding 10% (w v^{-1}) trichloroacetic acid (TCA) in 1:10 ratio (w v^{-1}). The homogenate was centrifuged at 15000*g* for 10 min and supernatant was collected. To aliquot of the supernatant (500 µL), 1.5 mL of 15% TCA containing 0.5% thiobarbituric acid were added. The solution was heated in a boiling water bath at 95 °C for 18 min and immediately cooled. The absorption of 1 mL aliquots of supernatant was read at 450, 532 and 600 nm. The MDA content was expressed as nmol (g DW)$^{-1}$ (DW = dry weight) and calculated in agreement with Bao et al. (2009).

Total soluble proteins were extracted by resuspending 1 g of frozen fruit tissue powder in 5 mL of extraction buffer 100 mM potassium phosphate buffer (pH 7.8), 1 mM sodium EDTA (pH 7), 5% (w v^{-1}) PVPP supplemented with 2 mM DTT, 1 mM PMSF, 0.2% Triton X-100. The homogenate was centrifuged at 18000*g* for 10 min at 4 °C and supernatant used for enzymatic activities.

Protein content for all examined crude enzyme extracts was measured by the Bradford assay (Bradford, 1976) using bovine serum albumin as a standard.

2.3. Biochemical analyses

PPO (EC.1.10.3.1) activity was determined following the modified method described by Chen et al. (2009). First, 2.5 g of fruit were homogenized in 5 mL of 100 mM sodium phosphate buffer pH 6.4 containing 0.125 g PVPP. Crude enzyme extract (100 µL) was incubated with a buffered substrate (500 mM catechol in 100 mM sodium phosphate buffer pH 6.4) in a final volume of 1.5 mL and monitored by measuring the increase in absorbance at 398 nm. The specific activity for molar change in catechol was expressed nmol (g DW)$^{-1}$

LOX (EC 1.13.11.12) activity was quantified following the method described by Pérez et al. (1999) with slight modifications. The enzyme was extracted by resuspending 1 g of frozen fruit tissue powder with 3 mL of extraction buffer (50 mM potassium phosphate buffer pH 7.8, 1 mM sodium-EDTA pH 7,2% PVPP). The reaction mixture consisted of 0.093 M sodium phosphate buffer pH 6, 0.17 mM linoleic acid sodium salt, and 50 µL of crude enzyme extract in a final volume of 1.5 mL. LOX activity was detected spectrophotometrically by recording the formation of hydroperoxides and the resulting increase in absorbance at 234 nm. LOX activity was expressed as the specific rate of molar change of hydroperoxides in µmol (g DW)$^{-1}$.

APX (EC 1.11.1.11) activity was assayed according to Garcia-Limones et al. (2002) with some modifications. The reaction mixture