Available online at www.sciencedirect.com
ScienceDirect
Postharvest Biology and Technology 42 (2006) 209–216
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Effect of high CO_2 pretreatment on quality, fungal decay and molecular regulation of stilbene phytoalexin biosynthesis in stored table grapes

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Received 17 March 2006; accepted 3 July 2006

Abstract

Table grapes (*Vitis vinifera*) cv. Cardinal stored at low temperature were analysed to determine the effect of pretreatment with 20 kPa O_2 + 20 kPa CO_2 + 60 kPa N_2 for 3 days on quality and control of decay. The pattern of stilbene synthase (STS) gene expression and trans-resveratrol levels were also analyzed in grapes during low temperature storage at 0 °C and further shelf-life at 20 °C for 2 days. Our results showed that high CO_2 pretreatment was effective for improving appearance of the bunches and maintaining the quality of the berries. In CO_2-treated bunches the browning and withering index, the decline in relative water content and the weight loss were also lower than in non-treated ones. The levels of *STS* mRNA and the accumulation of trans-resveratrol in CO_2-treated grapes were much lower than in the non-treated grapes during low temperature storage. Moreover, the pattern of *STS* gene expression and trans-resveratrol content in CO_2-treated grapes was consistent with the reduction of natural total decay produced by this pretreatment. This effective non-stressing treatment avoids the induction of trans-resveratrol during low temperature storage until its synthesis is enhanced during shelf-life at 20 °C.

Keywords: Table grapes; *Vitis vinifera*; Fruit quality; Postharvest technology; Carbon dioxide; Laser spectrometry; Stilbene synthase; Resveratrol

1. Introduction

Low temperature storage is one of the most effective technologies for extending the postharvest life of fruit and vegetables. However, in table grapes low temperature storage life is limited by high sensitivity to fungal attack, mainly from *Botrytis cinerea*. This pathogen, which has a considerable economic impact in horticulture, is usually controlled by means of fungicides, but this can lead to multiple resistance in the pathogen population (Raposo et al., 1996). Moreover, because postharvest chemical treatments are restricted in most countries, safe alternative control technologies need to be developed to assure high quality fruit and control fungal attack. Commercial alternatives to the use of SO_2 generators have been proposed to maintain the quality of table grapes over the short term, using modified atmosphere packaging (MAP) alone or in combination with natural fungicides (Artés-Hernández et al., 2006). Also, the application of controlled atmospheres (CA) under a continuous flow has been reported to be beneficial for controlling postharvest diseases in table grapes for prolonged cold storage (Yahia et al., 1983). However, rachis browning places a limitation on prolonged storage under CA (Crisosto et al., 2002; Retamales et al., 2003). An alternative gaseous treatment would be pretreatment with high CO_2 concentrations for shorter storage periods during postharvest handling of table grapes. Considering the low risk of this treatment, the use of pretreaments with high CO_2 levels to control *Botrytis* storage rot in maintaining table grape quality could be an interesting area of research. However, we need to know the efficacy of pretreatment with high CO_2 levels in controlling decay and maintaining quality of both rachis and berries. We also need to know whether this treatment corre-

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doi:10.1016/j.postharvbio.2006.07.002

lates with the activation of a number of defense mechanisms in grapes. In previous work, we reported that the expression of *Vitis* class I chitinase and β-1,3-glucanase genes is not enhanced with CO_2 treatments which control fungal decay, and we suggested that the efficacy of high CO_2 pretreatment on the reduction of fungal decay is not mediated by the induction of the above mentioned PR genes (Romero et al., 2006).

Among the other possible defense mechanisms, the induction of the phenylpropanoid pathway appears to play a crucial role (Hahlbrock and Scheel, 1989). In grapes, the production of phytoalexins such as stilbenes on the phenylalanine/polymalonate pathway (Langcake and Pryce, 1977) is one of the most important defense pathways. Phytoalexins from *Vitis* species are composed of a restricted group of molecules belonging to the stilbene family and deriving primarily from trans-resveratrol (3,4′-5-trihydroxystilbene). Trans-resveratrol, which has recently received a lot of attention because of its implication in human health (Bertelli et al., 1995; Jang et al., 1997; Hung et al., 2000), is mainly present in the skin of berries. Resveratrol production depends on many factors and the concentration is highly dependent on the cultivar. In grape tissues, trans-resveratrol production has been described after microbial infection or treatment with elicitors and several kinds of stress (Langcake and Pryce, 1976; Liswidowati et al., 1991; Jeandet et al., 1995). Specifically, production of trans-resveratrol in wine and table grapes in response to UV-C irradiation has been extensively studied (Creasy and Coffee, 1988; Langcake and Pryce, 1977; Cantos et al., 2002). Moreover, a good correlation has been reported between resveratrol production (as induced by UV-C elicitation) and grey mould resistance (Sbaghi et al., 1995), and also black mould caused by *Rhizopus stolonifer* on several table grape varieties (Sarig et al., 1997). Trans-resveratrol is synthesized by condensation of one molecule of 4-coumaroyl-CoA with three malonyl-CoA units in a reaction catalyzed by stilbene synthase (STS). This enzyme is encoded by a multigene family characterized by high nucleotide sequence homology (Melchior and Kindl, 1990; Wiese et al., 1994). A constitutive expression of *STS* has been reported (Sparvoli et al., 1994), but it could be expressed differentially in response to biotic and abiotic stresses (Preisig-Müller et al., 1999; Brehm et al., 1999; Versari et al., 2001).

The aim of this work was to analyze the effectiveness of pretreatment with 20% CO_2 in controlling natural postharvest decay and its effect on quality attributes of rachis and berries and the molecular regulation of stilbene phytoalexin biosynthesis. *STS* gene expression and trans-resveratrol content of table grapes cv. Cardinal during low temperature storage at 0 °C and further shelf-life at 20 °C for 2 days were monitored. These technological and molecular studies were based on the premise that an understanding of the role of high CO_2 levels in disease resistance and quality may contribute to the development of more effective postharvest technologies.

2. Materials and methods

2.1. Plant material

Table grapes (*Vitis vinifera* L. cv. Cardinal) were harvested at random in Camas (Sevilla, Spain) in July. Early-harvesting mature berries were used in this work (12.7% total soluble solids; 0.81% tartaric acid). After harvesting, field-packaged bunches were transported to the laboratory, where fruit were immediately forced-air precooled for 14 h at −1 °C. After cooling, bunches free from physical and pathological defects were randomly divided into two lots and stored at 0 ± 0.5 °C and 95% relative humidity (RH) in two sealed neoprene containers of 1 m^3 capacity. Ten plastic boxes containing about 3 kg of table grapes per box were stored in each container. One lot was stored under normal atmosphere for 33 days (non-treated fruit) and the other under a gas mixture containing 20% CO_2 + 20% O_2 + 60% N_2 (CO_2-treated fruit) for 3 days. The CO_2 concentration was maintained throughout the pretreatment experiment and was measured daily using an automated gas chromatograph system equipped with a thermal conductivity detector and Poraplot Q column (Varian Chrompack CP20033P). After 3 days, CO_2-treated grapes were transferred to air under the same conditions as the non-treated fruit until the end of the storage period. At the end of low temperature storage (33 days), both CO_2-treated and non-treated grapes were transferred to ventilated storage containers for an additional 2 days at 20 °C and 95% RH, to simulate shelf-life during marketing. Ten clusters were sampled periodically during low temperature storage and at the end of their shelf-life. Berries obtained from five clusters (approximately 300 g each cluster) were peeled and the skin was frozen in liquid nitrogen, ground to a fine powder and stored at −80 °C until analysis. For quality parameters, 45 berries were used, randomly removed from five clusters and distributed in three replicates of 15 berries each.

2.2. Quality assessments and total decay

Bunch withering, browning indexes and the relative water status (RWC) of the stem were determined for each bunch. Bunch withering was determined using the following subjective scale: (0) none, (1) onset of withering in pedicel and apex of stem, (2) withering of pedicel, apex of stem and over 10% of the main stem, (3) withering in up to 50% of main stem, and (4) total withering of main stem. The scale used for the browning index was: (0) none, (1) slight, (2) moderate, (3) severe, and (4) extreme. The water status of the stem was followed by measuring the RWC. One centimeter of stem, cut with a razor blade was weighed fresh, again after 24 h rehydration with distilled water at room temperature, and finally after drying at 85 °C, to give the fresh (FW), turgid (TW) and dry (DW) weights, respectively. RWC was determined from the equation: $RWC\ (\%) = (FW - DW)/(TW - DW) \times 100$.

Berry quality assessment considered dry matter (DM), soluble solids content (SSC), pH, titratable acidity (TA) maturity