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Postharvest Application of Sulphur Dioxide Fumigation to Improve Quality and Storage Ability of "Red Globe" Grape Cultivar During Long Cold Storage

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Table grape cv 'Red Globe' is produced in Sicily and in other areas of Southern Italy. This produce is very appreciated by consumers, having the best rankings in the world market for exports and quality. Nevertheless, despite it is a non-climacteric fruit, table grape is very perishable, both after harvest, and during postharvest handling and cold storage. In fact, also at low temperatures, table grape is very sensitive to fungal infection (*Botrytis cinerea*) and other diseases, like gray mold, which is very aggressive. For this reason, a standard practice is to fumigate table grape with sulphur dioxide (SO_2), after storage in chambers. The growing market request of table grape cv. 'Red Globe' enhanced postharvest research on better techniques aimed to maintain the grapes for longer periods, in order to satisfy the demand. Therefore, the object of this study is to assess the effect of cold storage, at 1±0.5 ºC and 93±2% relative humidity (RH), of table grape cv 'Red Globe', for 4 months, with low concentrations of SO_2 (0.20%), with the aim to extend their postharvest shelf life. Two samples of Red Globe table grape were stored at 1±0.5°C, the first for control without SO_2, and the other treated with low concentration of SO_2. Both samples were moved every 15 days, to 20 ºC for 3 days, for simulating shelf life and berry decay. Results showed that, 'Red Globe' table grape treated with low concentration of SO_2, prolonged its shelf life for three months after harvest. More particularly, firmness, weight loss, total soluble solids content and titratable acidity maintained good values, and rachis and berry decay were inhibited. This technique is a good alternative to the SO_2 one or dual release generating pads for maintaining grape quality in extended storage, with lower impacts for the environment and lower costs.

1. Introduction

Table grape is an important typical fruit of the Mediterranean diet. Its annual production in Italy is about 104×104 q, representing 15% of total world production. The 90% of Italian grapes are produced in Apulia and Sicily, cultivated on 24,690 and 15,900 hectares respectively. Producers own on average 2 hectares but in the Taranto, Catania, Caltanissetta and Agrigento provinces the figure is significantly higher reaching 4.22, 3.57 3.42 and 3.51 hectares/producer respectively (ISTAT, 2016).
Fruit quality at harvest depends on numerous factors such as climatic and soil conditions, cultivar, cultivation practices, degree of ripening and sanitary conditions. The postharvest life of table grape is limited by quality deterioration between harvest and retail, mainly due to weight loss, color changes and accelerated softening and ripening (Piazzolla, 2016). Moreover, several defects such as decay, berry cracking, stem browning, insect damage and grey mold infections may considerably affect the consumer acceptance.
Fruit of table grape (*Vitis vinifera* L.) are composed of two different structures, the edible berries and the rachis, that provide structural support and solute transport to the grape clusters. One of the most important visual quality parameters of table grape clusters is the rachis color. Immediately after harvest, rachis undergoes a rapid visual detriment referred to as rachis browning (Silva-Sanzana, et al. 2016). This situation affects overall cluster quality and has been associated mainly to water loss (Crisosto et al., 2001, Lichter et al.,

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2011 and Valverde et al., 2005) and oxidation processes (Carvajal-Millán et al., 2001). However, previous studies suggest that other cultivar dependent factors could be involved (Crisosto et al., 2001). Grape is a non-climacteric fruit and does not ripen further after harvest, so harvesting at the proper stage of maturity is essential for optimal grape quality in terms of soluble solids, berry weight, titratable acidity and overall sensory characteristic (Gómez et al. 1995). Grape ripening is a physiological period that starts at the moment of veraison and lasts until the fruit is harvested. This is a very important period that influences grape composition and determines varietal characteristics. Because table grape is a non-climacteric berry, with a low rate of physiological activity, it is very sensitive to water loss and gray mold (*Botrytis cinerea*) during postharvest handling and cold storage (Palou et al., 2010; Valero et al. 2006). Gray mold is the most aggressive postharvest disease, because of its ability to develop at low temperatures (Liguori et al. 2015b). Table grapes are stored at −0.5°C and RH of 95% for 40–100 days depending on the cultivar (Zoffoli and Latorre 2008). Commercial storage relies on the sulfur dioxide (SO_2) technology, which is applied during storage from slow-release pads. While consumers, generally, associate a green rachis with freshness, when they encounter bunches with a brown rachis, they cannot know if the grapes were harvested 1 week or 2 months prior, and they are likely to categorize the grapes as unattractive. Therefore, rachis browning serves as a freshness marker, and it is likely to play a major role in consumer preference and food waste.

The use of conventional synthetic fungicides for controlling pathogens on most commodities is prohibited after harvest in most EU countries. In grapes and some other fruits, however, the use of sulfur dioxide during storage is permitted, since it is considered as processing aid and not as a fungicide. Sulfur dioxide can damage the fruit by causing surface cracks and bleaching color from red cultivars (Luvisi et al., 1992).

To achieve good levels of control, usually SO_2 is applied in storage room of grapes weekly, following a first treatment during cooling prior to cold storage and/or grapes are packed with pads releasing sulfur dioxide (Luvisi et al., 1992 and Leesch et al., 2014). The dual-phase release SO_2 pads showed better performance for the long-term storage of grapes than single-release pads (both as regards of berry sensory attributes and stem appearance, with lower stem browning). The dual-phase release SO_2 pads extended the shelf-life of grapes by around 1 month, depending on the cultivar Fernández-Trujillo,et al., 2012).

The purpose of this study was to assess the effect of cold storage, at 1±0.5 ºC and 93±2% relative humidity (RH), of table grape cv 'Red Globe', for 4 months, with low concentrations of SO_2 (0.20%), in order to extend the postharvest shelf life of fruit.

2. Materials and Methods

2.1 Plant material and experimental design

This study was conducted during the 2013 season on 10-year-old table grape plants, *Vitis vinifera* L. cv. 'Red Globe', in a commercial vineyard located in the Central Sicily (Southern of Italy) near Agrigento (37°18'25.0"N 13°52'11.0"E 300 m asl). Grape plants were supported on an overhead arbor 2 m high 'Tendone system' covered with netting and plastic film (LDPE, thickness 170 µm), spaced at 2.5m×2.5m (1600 plant ha^{-1}).

The bunches were harvested at a stage of maturity and time normally optimal for commercial harvest. Once in the laboratory, the berries were detached from bunches, selected for uniformity in size, ripeness and absence of defects, and were processed the same day of harvest (Sortino et al., 2017). Grapes were subjected to a fast cooling for 12 h in forced air tunnels at 0 ºC to lower berry temperature to 1–4 ºC and were, then, stored in 200 mq^2 chamber for 120 d at 1±0.5 ºC RH 92±3 % and 0.07 m/s air velocity. After cold storage, grapes were maintained for 3 days at 20 ºC, to simulate a commercialization period before the incidences of decay were determined (Sortino et al., 2015). The temperature and relative humidity of the cold rooms were examined by placing a Hobo data logger for a week in room (Onset®-HOBO® Massachusetts, U.S.).

2.2 Fumigation chamber

The storage chamber has efficient, safe and economical fumigations. The fumigation room that we used for this research was prepared so as to be: gas-tight; equipped with an efficient system to apply and distribute the fumigant; providing an efficient system for the removal fumigant at the end of treatment; equipped with a visual alarm for the staff who work with or near the room; provided of a gas and control of the semi-automatic precision injection system; with gas evacuation system, small window to control the room and the SO_2 level control system. The semiautomatic SO_2 precision generator used in this research was provided by Fruit Control Equipments srl, Milan, Italy.

2.3 Physical and chemical quality evaluation

During storage, fruit samples were taken at 15 days intervals from different storage/treatments (Control – CTRL- /0.20% SO_2) conditions and various physical and chemical analyses on the fruit were performed during the entire storage. Flesh firmness was measured by Turoni, model 53200, Forlì (Italy) and values were

expressed as N. Titratable acidity was determined by titrating 2 ml of fruit juice in 38 ml of distilled water with 0.1 N NaOH to the end point of pH 8.1 and expressed as percent of tartaric acid equivalents (Crison Instruments, Titromatic 8652 Spain). Total soluble solids content were determined using a hand refractometer (Palette PR-32, Atago Co., Ltd-Tokyo, Japan) and means were expressed as percentage (%).
Berry color was determined with a Minolta Chroma Meter CR-400 (Osaka, Japan). Color measurements were recorded using the CIE L*a*b* color space. From these values, hue angle was calculated using the following equation: $h^{\circ} = \tan{-1}(b^*/a^*)$. Color values were obtained for 50 berries per replicate. Two measurements were taken from opposite sides at the equatorial region of each berry. Rachis browning was expressed as a percentage of berries having brown rachis. SO_2 levels inside the chamber were monitored weekly using an electrochemical sensor with a small pump to extract air from the package into the GA25 analysis WIKA Alexander Wiegand SE & Co. KG Germany. The instrument was connected to tubes attached to each replicate boke so that air was drawn into the instrument and returned to the package. Sulfite content in the berries was determined according to the optimized Monier-Williams distillation method (Lichter et al., 2008).

2.4 Sensory evaluation

The boxes (10 kg) were transferred to 20 ºC, 80±5% RH for 3 d, subsequently 10 bunches from each box were examined individually (Lichter et al., 2005). Each bunch was rated for desiccation, SO_2 damage, and visual quality. Desiccation and SO_2 damage were assessed on a 5-pt scale. Desiccation of rachis and of pedicels scores were 1 = green as at harvest; 2 = slight browning; 3 = browning but no shriveling; 4 = browning and some shriveling; and 5 = dry and brown. Scores above 3 were considered unmarketable. SO_2 ratings were defined according to the number of berries per replicate that suffered bleaching: 1 = no apparent bleaching; 2 = two to five berries; 3 = six to 10 berries; 4 = 11 to 20 berries; and 5 = over 20 bleached berries per 10 bunches. Healthy bunches were defined as having only one or no decayed berries, and the percentage of such bunches in each box was determined. Visual quality of bunches was evaluated using the following scale: 5 = excellent, no defects; 4 = very good, minor defects; 3 = fair, moderate defects; 2 = poor, major defects; 1 = inedible. A score of 3 was considered to be the limit of marketability and a score of 2 the limit of edibility. The evaluation test was performed by an evaluation team consisting of nine panelists (five men and four women, 25-60 years old) with a good background and knowledge of the details of this evaluation and trained about table grape for one week. During the evaluation, all nine panelists completed a short questionnaire covering the quality indicators independently (Allegra et al., 2015). The evaluation was carried out in a special room with individual booths under white lights. Samples were presented in a white plastic plate and tasted 1 h after they were taken out of the cold room (Liguori et al., 2015a).

2.5 Statistical analysis

The study was planned with randomized sampling design. Statistical differences with P-values under 0.05 were considered significant. The Tukey test was used for comparing the averages of measured values.
Data for the physical, chemical, and sensory parameters were subjected to analysis of variance. Sources of variation were time of storage and treatments. Mean comparisons were performed using the Tukey HSD test to examine if differences between treatments and storage time were significant at $P < 0.05$. All analyses were performed with SPSS software package v.20.0 (IBM-SPSS Inc. 2001) for Windows.

3. Results and Discussion

With regard to the weight losses, 'Red Globe' grape stored with SO_2 maintained good values, more particularly, the weight losses after 120 days of storage was 2.86% (Table 1), contrarily, for control results show a weight loss of 4.18% at the same stage. Effects of storage times on weight losses were statistically significant ($p<0.05$). In respect of flesh firmness, the effect of SO_2 treatment was likewise positive; after 90 days of storage berries were firmer and more marketable than control, and, at the end of storage period (120 days), grape treated with SO_2 reported the highest values of flesh firmness (23.5 N), contrarily, the lowest value was obtained for CTRL (20.91 N). It is notable that grape at harvest has a value of flesh firmness of 30.5 N which decreased to 20.9 N at the end of storage (Table 1). Also in this case, effects of storage periods, on flesh firmness, were found statistically significant ($p<0.05$). After 120 days of storage with SO_2 treatment mean value of total soluble solid content of grape was 14.51% and 13.67% for control. After 90 days grape stored with SO_2 had the highest soluble solids, contrariwise, the lowest values were observed in the control. Storage periods influenced significantly ($p<0.05$) the content of soluble solid in tested grape (Table 1). Titratable acidity of grapes is shown in Table 1. Titratable acidity at harvest was 0.46 and decreased to 0.34% at the end of the storage period (120 days), during storage, the highest titratable acidity was observed for grape stored with SO_2 (0.37%). Berry color L* values decreased and h◦ values increased during storage regardless of the treatment (Table 1). The L* and h◦ values were 32.89 ± 1.10 and 13.85±0.33, respectively, at harvest and

28.46 ± 0.78 and 19.49 ± 1.11 at the end of storage with SO2 treatment, and 28.05±0.55 and 20.94±1.15 at the end of storage for CTR.

Table 1: Physical and chemical behavior of grape cv 'Red Globe' during the long cold storage of 120 days

Days		Weight	SSC	Titratable	Firmness (N)	Berry color	
0	SO_2	0.0±0.0d	15.30±0.21b	0.46± 0.05ns	30.5± 0.16a	32.89±1.10ns	13.85± 0.33d
	CTRL	0.0±0.0d	15.30±0.21b	0.46± 0.05	30.7± 0.16a	32.89± 1.00	13.85±0.45d
30	SO_2	2.57±0.11c	15.51±0.14b	0.44±0.05	30.2±0.20a	31.85± 1.25	15.51±0.81c
	CTRL	3.00±0.10a	15.38±0.18b	0.43 ±0.02	26.2±0.57b	31.78± 1.26	16.39± 0.99c
60	SO_2	2.69±0.05 b	15.00±0.12b	0.42±0.09	29.2±0.25a	30.21± 0.97	16.64±0.67c
	CTRL	3.54±0.14a	14.70±0.14b	0.40±0.06	25.5±0.21c	31.53± 1.17	17.51±1.07bc
90	SO_2	2.82±0.19b	14.61±0.17b	0.37±0.04	23.5±0.24c	29.62± 0.88	17.89±1.0b
	CTRL	3.90±0.13b	14.25±0.14b	0.35±0.07	21.6±0.12d	29.05± 0.94	18.44±1.09b
120	SO_2	2.86±0.16b	14.51.±0.11b	0.37±0.04	23.0±0.78c	28.46±0.78	19.49±1.11ab
	CTRL	4.18±0.25a	13.67±0.13b	0.34±0.08	20.9±0.12d	28.05±0.55	20.94±1.15a

Table 2. - Quality measurements of 'Red Globe' after storage at 1±0.5ºC and 3 days at 20 ºC.

	Rachis desiccation (1-5 scale)[(1)]				Pedicel desiccation (1-5 scale)[(1)]			
Day	Storage	Storage	Shelf life [(y)]	Shelf life [(y)]	Storage	Storage	Shelf life	Shelf life
0	1.0±0.0c	1.0±0.0b	1.1±0.1c	1.1±0.1d	1.0±0.0d	1.0±0.0d	1.0±0.0d	1.0±0.0d
15	1.2±0.2c	1.1±0.1b	1.4±0.2c	1.1±0.1d	1.0±0.1d	1.0±0.1d	1.0±0.0d	1.0±0.1d
30	1.5±0.3c	1.3±0.7b	1.8±0.4bc	1.4±0.3c	1.4±0.2c	1.5±0.3c	1.3±0.5d	1.5±0.5c
45	1.5±0.3c	1.2±0.5b	2.1±0.4b	1.5±0.3c	2.6±0.3b	1.5±0.8c	2.6±0.2b	1.6±0.4c
60	2.1±0.5b	1.5±0.2b	2.4±0.7b	1.7±0.2b	2.5±0.4b	1.9±0.6c	2.6±0.7b	2.2±0.3bc
75	2.4±0.3ab	1.9±0.1ab	2.4±0.6b	1.9±0.5b	2.9±0.3b	2.4±0.2b	2.7±0.4b	2.5±0.2b
90	2.7±0.8ab	2.0±0.3a	2.8±0.6b	2.2±0.2b	2.7±0.6b	2.3±0.5b	3.0±0.1b	2.5±0.2b
105	3.3±0.7a	2.4±0.3a	3.6±0.4a	2.5±0.3a	3.0±0.4b	2.5±0.4b	3.8±0.6a	2.4±0.3b
120	3.5±0.5a	2.3±0.5a	3.7±0.5a	2.6±0.3a	3.8±0.4a	2.9±0.5a	4.4±0.4a	3.2±0.4a

[(x)] Storage time was 120 days at 1 °C. Quality measurements included rachis and pedicel desiccation, SO_2 damage, and the percentage of bunches with one or no decayed berries at the end of storage. [(y)] Shelf life after 3 days at 20 °C once table grape was stored for each stage. [(1)]1 = rachis and pedicels fully green as at harvest; 2 = slight browning; 3 = browning of rachis and pedicels but no shriveling; 4= browning and some shriveling; 5= both rachis and pedicels dry and brown. A rating greater than 3 was considered unmarketable. Values in columns followed by different letters indicate significant differences according to analysis of variance with the Tukey test at $P < 0.05$.

Similarly, Artes-Hernandez et al. (2004) reported a decrease, during storage, in L* values, due to water loss of berries which reported, also, no changes in h◦ values for 'Autumn Seedless' grapes unwrapped or wrapped with OPP film after two months of cold storage. In this study, marked color changes were observed after 30 days of storage. Mlikota Gabler et al. (2005) reported increases in h◦ values, which indicate a progression in berry color toward brown, but the increases that they reported are not visible to the naked eye. In the present study, berry browning was not visible in the treatment with SO_2. During 4 months of storage, lower h◦ values were found in CTR. Table 2 shows rachis desiccation scores given to grape by panellists. Again, in this case, the effects of storage periods on rachis desiccation were found statistically significant ($p<0.05$). The rachis desiccation scores increased from 0d to 120d and the highest value (3.5) was observed in the clusters stored as CTRL, contrarily the lowest value (2.3) was observed for grape treated with SO_2 after 120 days. Pedicel desiccation value (Table 2) at harvest was 1 (rachis and pedicels fully green as at harvest) and 3.8 at the end of the storage period (120 days). After 120 days, the value of simulated shelf-life, after 3d at 20°C, increased up to 0.6 points for CTR (pedicel value 4.4) and up to 0.3 points for the SO_2 treatment (rachis 2.6 and pedicel 3.2). The method applied in this study minimizes water loss leading to rachis desiccation, and prevents both decay which occurs if SO2 is too low, and SO2 damage which occurs if SO_2 is too high.

During the entire storage, the best score was obtained from the grapes stored treated with SO_2. The scores of SO_2 damage and healthy bunches (%) values obtained during the storage were very good until 90 days (Table 3). The colour changes of berry during the storage period of 4 months showed significant difference among all stages. The berries with SO_2 treatment showed a better visual quality until 105 days of storage (Table 3).

Table 3. - The effect of SO_2 treatments on the development on cold stored table grapes after storage at 1±0.5ºC and 3 days at 20 ºC.

	Visual quality(*)	SO_2 damage (1-5 scale)(1)	Healthy bunches (%)
Days		Storage	Shelf life
0	5.0±0.0a	1.0±0.0b	0±0.0c
15	4.9±0.1a	1.0±0.0b	0±0.0c
30	4.5±0.1b	1.0±0.0b	0±0.0c
45	4.1±0.2b	1.0±0.0b	0±0.0c
60	4.0±0.3b	1.0±0.0b	78±15a
75	3.8±0.5c	1.2±0.1b	71±13b
90	3.5±0.3c	1.5±0.5b	75±28a
105	3.1±0.2cd	1.9±0.3b	100±0a
120	2.9±0.2d	4.0±0.5a	100±0a

(1)Damages rating according to the number of berries per replicate (10 bunches) that suffered bleaching: 1 = no apparent bleaching; 2 = two to five berries; 3 = six to 10 berries; 4 = 11 to 20 berries; 5 = over 20 bleached berries. Values in columns followed by different letters indicate significant differences according to analysis of variance with the Tukey test at $P < 0.05$.
(*) Five point scale: 5 = excellent, no defects; 4 = very good, minor defects; 3 = fair, moderate defects; 2 = poor, major defects; 1 = inedible

4. Conclusions

The low concentration SO_2 treatment was more effective throughout storage in regards to incidence of fungal decay, stem browning, and visual appearance. The results presented here show that SO_2 fumigation appears to be a very promising economic technology to reduce decay and increase the shelf life of 'Red Globe' grape. In addition, repeated fumigations every week during cold storage in four months has significantly improved the degradation control. Moreover, our results show that SO_2 fumigation was, to some extent, able to kill pathogens on the surface of fruits that cause decay in favorable conditions. The storage shelf life of cv 'Red Globe' was limited to 4 months. After 4 months of storage, very marked color changes were observed, more than 90% fungal decay occurred, severity of stem browning increased, and appearance was unacceptable with the absence of SO_2 treatment. To our knowledge, little has been done in the use of low dosages SO_2 optimized for each cultivar to extend the marketing life of table grapes, so future efforts should develop in this direction. Currently, intensive work has been developed by our group for the use of SO_2 and SO_2 alternatives as a means to extend the shelf life of table grape, and other fresh fruit or minimally processed fruit.

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