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Research Article

Post harvest immersion of grapes in ethanol and their quality assessment

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Abstract

Postharvest deterioration of table grapes commonly results from berry decay and/or desiccation of stems and pedicels. The standard method to control postharvest decay of cluster grapes is to fumigate the fruit instantaneously after harvest with sulfur dioxide gas. However, it results in fruit damage, unpleasant aftertaste and allergies. Our objective was to observe the effect of post harvest ethanol rinse including packing in perforated plastic box, alternatives to sulfur dioxide to sustain quality and inhibit grey mold. Grapes were sorted but keeping the clusters intact. After initial preparation, they were subjected to ethanol wash with no and minimal heating (30^0C,) and packed in perforated plastic box. The treated grapes as well as the control grapes were stored at 0 °C for up to 3 weeks. Ethanol dip along with gentle heat treatment resulted in significantly ($P < 0.05$) maintained firmer texture and total sugar, higher overall visual quality, lower decay rate, and weight loss than control and other treatment during entire storage period of 21days.

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Keywords: sulfur dioxide, ethanol, post harvest technology, table grapes, total sugar, firmness

Introduction

Grapes (*Vitisvinifera L.*) are a well perishable commodity and their market life is a function of time and temperature, with the degree of deterioration related directly to the duration of exposure to higher temperatures. They are non-climacteric fruits which are meant for consumption as fresh with washing. Quality of table grapes is usually considered as a combination of appearance and flavor during shelf-life. A decrease in quality during postharvest handling is generally associated with water loss, decay causing fungi, browning of the cluster stem and shelling of berries. Gray mold rots emergent in grapes over the postharvest period are causing severe losses. Solutions to limit pre-harvest treatments with synthetic fungicides are of particular interest to the grape industry as chemical residues are restrictive access to international markets. [Nigro et al., 2006].

Infections that cause postharvest losses can originate from spores on the surface of the berries, microscopic latent infections that occurred before harvest during the growing season, or visibly infected berries during packaging. [deKocket al., 1994]Length of storage is therefore limited by certain factors. In such conditions, handling, packing and special cooling methods are essential for the delivery to the consumer may range from some days to a month. High relative humidity is necessary to reduce water loss, in addition, low temperature is indispensable also to reduce the respiration rate. Improvements have resulted from advances in procedures of pre-cooling, judicious use of sulphur dioxide, better temperature and humidity control and more appropriate handling of grapes. [Burger et al., 2005]

Among post-harvest fungal pathogens, *Botrytiscinerea* is one of the common causal agents of grey mould of grapes. It can develop in the vineyard and even more after harvest, during long-distance transport, cold storage. The grapevine industry is particularly affected

by this fungus that attacks grapes at pre- and post-harvest stage under a wide array of environmental conditions and over a large geographical area. Infections caused during post-harvest conditions lowers the shelf life and adversely affect the market value of the fruits. Fungicides that are primarily used for controlling post-harvest diseases have recently come under special scrutiny as posing a potential on-cogenic risk. Therefore, the scientific community at international level is looking for safer substitute. [Liping et al., 2009; Tripathi et al., 2008]

Previously, postharvest diseases of table grapes were wieldy controlled by the application of SO_2, either by weekly fumigation in storage rooms or by packing grapes in polyethylene-lined boxes with SO_2 generator pads. Problems associated with this chemical use include the following:

(1) SO_2 residues that exceed the tolerance of 10 mg/kg

(2) Unsightly bleaching injuries that can occur to berries after numerous or high dosage fumigations

(3) Susceptibility of some people to sulphite allergies, the dietary danger of SO_2 was renowned, and there are concerns about the carcinogenic effect of long-term ingestion of the residues left on the fruit on consumption.

The dietary hazard of SO_2 was recognized and it was removed from the US Food and Drug Administration GRAS classification in 1986 [Zahavi et al., 2000; Lichter et al., 2002]. The standard method to control postharvest decay of cluster grapes is to fumigate the fruit immediately after harvest with sulfur dioxide gas followed by additional sulfur dioxide application during storage using either direct treatment or fumigation through continuous-release SO_2 generating pads. However, the concentration of sulfur dioxide necessary to inhibit fungal growth may induce injuries in both rachis and berries [Crisoto et al., 2002]. In addition, sulfite residues pose a health risk for allergic individuals and its applications have been restricted in many countries [Lurie et al., 2006]. For grape fumigation, repeated applications of SO_2 was necessary because the treatment kills the fungal mycelia and spores present only on the berry surface, but do not affect internal Botrytis infections that may lead to gray mold nesting.

However, it has been shown that excessive levels of SO_2 can damage table grapes by bleaching or causing sunken areas on the berry surface or contributing to premature browning of the stems. Therefore, safe alternative technologies are needed to control fungal growth and assure high-quality fruit. By applying a moderate heat treatment, ripening could be delayed and fungal decay reduced devoid of major changes in fruit quality. [Cantín et al., 2011). Ethanol and potassium sorbate are common food additives with potent antimicrobial activity. Ethanol dips and vapors have been reported to control postharvest diseases of peaches, citrus fruit, and table grapes. Ethanol application is considered a good substitute for the use of fungicides for controlling postharvest microorganisms. This compound is considered a GRAS (Generally Recognized as Safe) [Gutiérrez-Martínez et al., 2012].

Immersion of detached berries in 70% ethanol eliminated most of the fungal and bacterial populations on the berry surface, but had little effect on survival of yeasts. Dipping of grape bunches in 50, 40 or 33%, but not in 20% ethanol, prior to packaging, resulted in inhibition of berry decay that was equivalent to, or better than that realized with SO_2, released from generator pads. The use of higher concentrations of ethanol incurs additional ethanol costs and exacerbates safety hazards and disposal concerns that can reduce the feasibility of ethanol us. [Karabulut et al., 2005]

Decay control was generally feasible for a cold storage period of 4–5 weeks and sometimes more. Ethanol did not impair bunch appearance, berry bloom or berry firmness and ethanol-treated berries obtained higher organoleptic scores than SO_2-treated berries. [Lurie, 1998]Reducing ethanol by 10 to 20% and combining it with chitosan can kill spores of B. *cinerea*rapidly, while those 20% and below were sub lethal. [Romanazzi et al., 2007].

The aim of this study was to verify the worth of Ethanol alternative to sulfur dioxide (SO_2) in maintaining quality and reducing fungal decay during cold storage of Table Grapes.

Materials and Methods

Plant material

Grapes (Gola Variety) were collected from Vineyard of Bari, Chakwal, after harvesting immediately transported to Post Harvest Research Center (AARI), Ayub Agriculture Research Institute, Faisalabad, Pakistan. Grapes were washed and sorted for defects and randomly divided into three groups:-

- T_0 Control grapes
- T_1 Grapes immersed in 35%V/V Ethanol at 20°C
- T_2 Grapes immersed in 35%V/V Ethanol at 30°C

Storage and Quality parameters study

All treatments were surface dried, packed in polyethylene bags of 0.22mm thickness and stored at 0°C with 90-95% relative humidity up to maximum marketable life. Following physiochemical analysis of all treatments was done during storage after each three days interval. The six fruits were randomly grouped in three samples of two units; the pulp was obtained separately, homogenized and used for subsequent analysis.

Weight loss (%)

Selected fruits of each treatment were weighed using electronic digital balance (YAMATO Scale Co., Ltd, Japan). Weight loss was calculated by using following expression:-

$$\text{Weight loss \%} = \frac{\text{Weight of fruit at first interval - Weight of fruit at second interval}}{\text{Weight of fruit at first interval}} \times 100$$

pH

pH of grapes was recorded using single probe digital pH meter (Spectrum).

TSS

Total Soluble Solid (TSS) content of grape juice was determined by Abbe's Refractometer (Atago Pocket PAL-1, Tokyo, Japan) by placing a drop of pulp on its prism. Percentage of TSS was noted from direct reading of the refractometer.

Total Sugar

Stock solutions of glucose, fructose and lactose (4.0 g l−1) were prepared by dissolving each of the crystalline compounds in distilled water. Standard solutions of these reducing sugars or their mixtures were diluted to the required concentrations in 10 ml volumetric flasks. Stock solutions of potassium ferricyanide ($K_3Fe(CN)_6$, 4.0 g l−1) and sodium hydroxide (1.5 mol l−1) was prepared by dissolving the appropriate amount of each compound in distilled water. Date pulp was extracted in a juicer, and then 10 ml of the juice was placed in a 25ml volumetric flask. For spectrophotometric analysis, the analytes and any other reagents were added directly to a 10mm cell by using micropipettes. Taking into account that the total useful volume was 2.7 ml, 0.7 ml of 1.5 mol l−1 sodium hydroxide and 1.4 ml of double distilled water were initially mixed; then 0.4 ml $K_3Fe(CN)_6$ solution were added to give a volume of 2.5 ml, and finally, 0.2 ml of standard solution of a reducing sugar (or a mixture of the sugars) was micropipette to give a total volume of 2.7 ml. The mixture was then stirred as rapidly as possible by a hand-controlled micro-stirrer. After 30 s, the cell containing the mixture was put into the cell stand, which will kept at a constant temperature of 80◦C in the thermostated water bath. The absorbance was recorded at 420 nm with respect to the distilled water as blank. [Yongnian, Huang and Kokot (2003]

Decay %

Percentage decay was scored as visible fungal appearance by evaluating 2 fruit clusters randomly selected per replication per treatment.

Firmness

Firmness was measured, with a digital Penetrometer (tr, 53205, Forli, Italy). Results were reported as kilogram.

Data analysis

Statistical analysis was performed using one way ANOVA followed by LSD using Duncan Multiple Range test (DMRT) in software Statistix 8.1.

Results

Data of analysis of variance of the studied traits under the effects of ethanol treatments, storage periods, and their interaction are summarized in Table I. Significant effects of ethanol is obvious from table on pH, soluble solids concentration, Acidity, weight loss, total sugar firmness at $p \leq 0.01$, but no significant effects were shown on titratable acidity. (Fig. 1, 2, 3, 4, 5, 6)

Results pertaining to weight loss in this experiment show varying behavior of treatments and control grapes stored in same environment. Loss in mass was obvious in control grapes with 11.25% as compared to treatments. Grapes treated with 35%V/V ethanol at 30°C showed lowest loss in mass from 0 to 5.99%. Total weight loss was highest when fruits were treated with 30% v/v ethanol at 30°C compared to other treatments.

Table 1: Means of physcio-chemical parameters of Grapes treated with ethanol

Treatment	pH	TSS (%)	Acidity (%)	Total Sugar (mg/100g)	Firmness (kg)	Wt. loss (%)
T0	3.18c	22.65a	0.98a	20.85a	0.27c	6.78a
T1	3.29b	21.31b	0.89b	19.53b	0.33b	3.29b
T2	3.36a	20.74c	0.87b	19.50b	0.40a	2.75c

Means carrying same letters are statistically insignificant

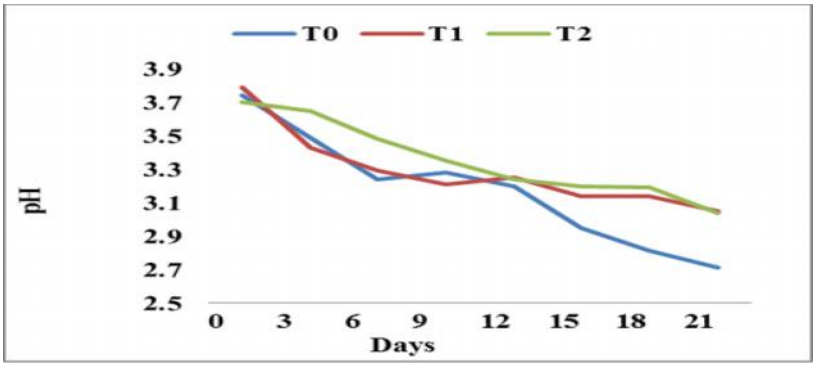

Fig.1 pH of fruits during storage

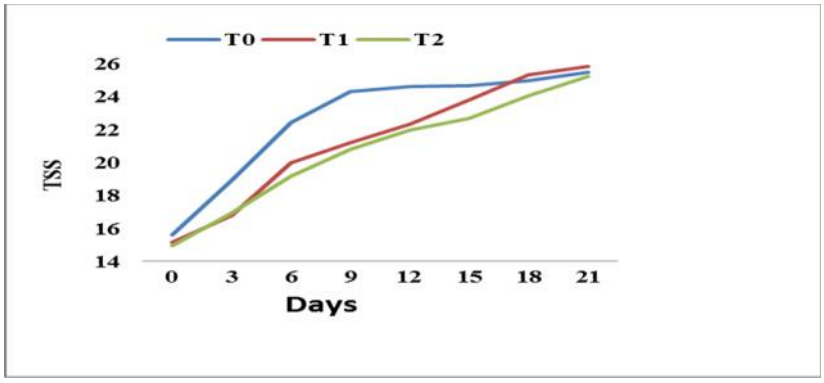

Fig.2 TSS of fruits during storage

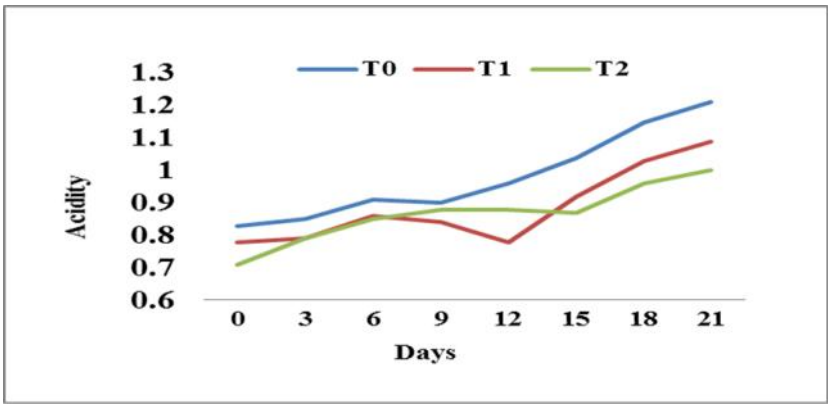

Fig.3 Acidity of fruits during storage

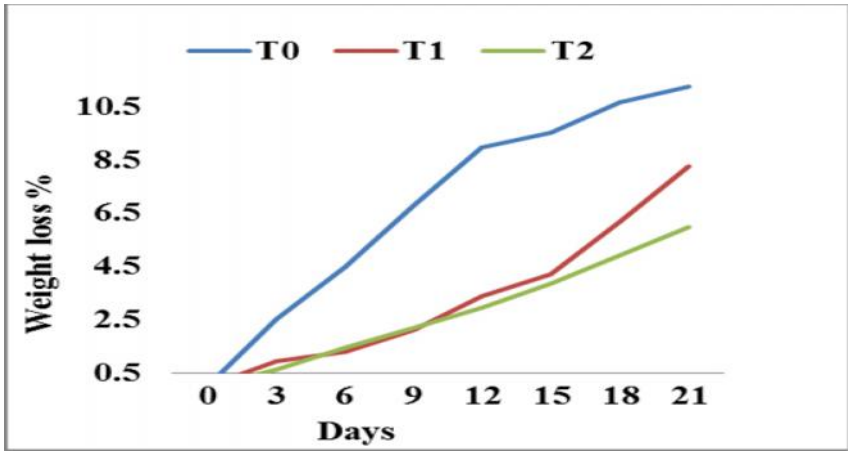

Fig.4 Weight loss % of fruits during storage

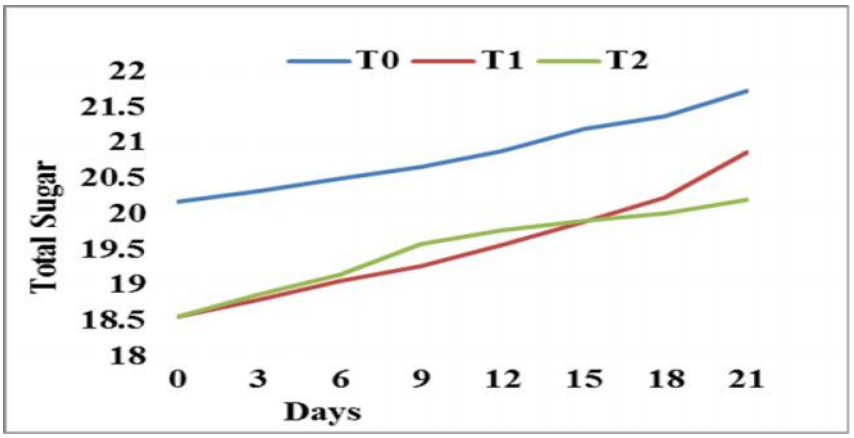

Fig.5 Total sugar % of fruits during storage

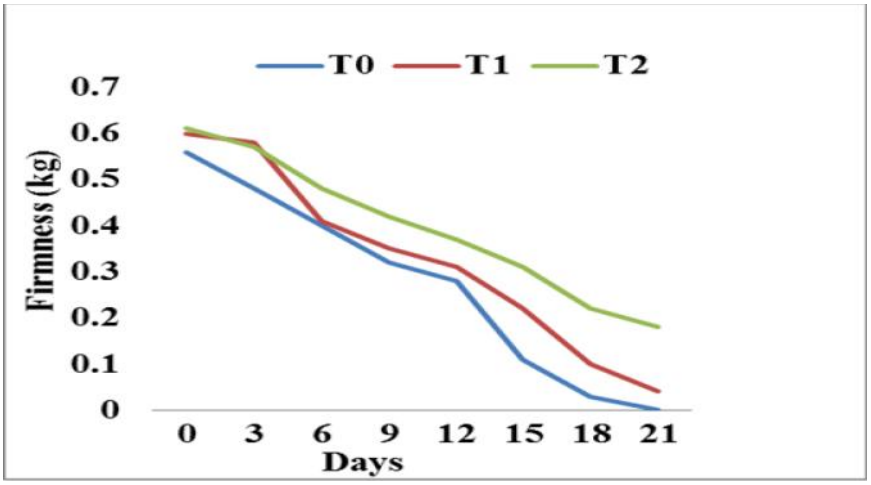

Fig. 6 Firmness of fruits during storage

Decay percentage increased as storage time increased for all treatments. At 9 and 21 days of storage, grapes treated with ethanol had less decay compared to control at the same times of storage.

Significant reduction in fruit firmness over the storage time for all the treatments has been shown (Table 3). Comparing the control vs. the two other treatments, differences are prominent after 9 and 14 days of storage. However, at 3, 6 days of storage, fruits treated with 30% v/v ethanol at 30°C stored at 0°C were significantly firmer than control treatment but did not significantly differ from fruits treated with 30% v/v ethanol at 20°C treatment.

Juice pH significantly increased as the storage time increased; however, it varied slightly in significant between treatments. This variability was within a narrow range of 0.27 pH units. At 14, 18 and 21 days of storage, juice pH was significantly higher in control than others treatments.

Soluble solid concentration (SSC) increased over the storage time for all treatments, with the highest being (21.44) for the control treatment by the end of the storage periods (Table 1). Differences in soluble solid concentration between treatments and the control were shown in approximately18, and 21 days of storage, in which soluble solid concentration was significantly higher in the control treatment than fruits treated with 30% v/v ethanol at 30°C but not significantly differ from fruits treated with 30% v/v ethanol at 20°C.

Titratable acidity (TA) increased with storage duration for all treatments (Table 3). The differences in treatments showed an increase in titratable acidity in fruits treated with 30% v/v ethanol at 20°C at 7 and 21 days compared to control, but did not differ from fruits treated with 30% v/v ethanol at 20°C. Initial SSC content of grape berries was 17.7ºBrix.

Over the experiments, the grapes did not present variations in titratable acidity or refractometric index. Noticeable limitation in natural incidence of decay, most of which was gray mold (75–80%), was significantly reduced in fruits treated with 30% v/v ethanol at 30°C then others.

During storage, independently of the treatment carried out, an increase in the browning of the pedicels and a decrease of the fruity flavour and of the berry hardness and crispness occurred.

Discussion

The rate of loss of moisture from fresh fruits is largely dependent on the humidity and temperature of the surrounding air, as well as on the heat and mass transfer properties of the fruit. Grape berries don't show symptoms of water loss until the damage is quite evident on the stems. At about 4-5% mass loss, berries feel soft and above 5% loss in mass the wrinkles start to appear. However, despite good temperature control during postharvest storage, table grapes continue to lose mass mainly due to the micro-climatic conditions that were created within the enclosed fruit packages. After 75 days of cold storage the maximum mass loss of berries was less than10%, while the maximum mass loss of stems was in the range of 49.2± 4.66% [Ngcobo et al., 2012]. Results of this experiment support previous findings that ethanol treated grapes have beneficial effects in suppressing decay during the storage time. [Ngcobo, 2013]

Fruit softening is a biochemical process, normally attributed to the deterioration in cell wall composition that involves the hydrolysis of pectin by enzymes. Low levels of oxygen and elevated levels of carbon dioxide restricted the activities of these enzymes and allowed retention of the fruit firmness during storage. Moreover, water counted and the rachis appearance was rated. Gray mold infected grapes were identified by the characteristic slip-skin symptom and sporulation. [Feliziani, 2012]

Treatment with ethanol vapor or hot air alone both resulted in significantly lower decay incidence compared with the control. [Wang et al., 2011]. Gray mold incidence after 1 month storage decreases when fruit are immersed in the combination of 35% ethanol at 50°C for 1 min. A highly significant interaction (p _ 0.001) between ethanol and temperature was observed on percentage infection. As ethanol concentration and temperature increased, less infection was observed. Compared to the control treatments where fruit had a 100% infection, there was complete control over infection in those treatments of 300 ml/L ethanol and 50°C of temperature. [Gutiérrez-Martínez, 2011]

Fruit firmness decreased during the 12-day storage period. During storage of mango fruits pH values ranged from 3.5 to 4.5. The highest pH value of 4.5 was obtained in fruits treated with 300 ml/L of ethanol at 45 and 50°C. Control fruit showed a pH value of 4.2 at the end of the storage period. Likewise the collective

application of ethanol and heat to control pathogenic microorganisms has been well documented in temperate fruits such as table grapes, lemons, peaches and nectarines.

Results are in –line with conclusion of [Karabulut et al., 2009] who claimed that chlorine dioxide, hydrogen peroxide is a strong disinfectant and has broad antimicrobial activity inhibit postharvest decay of table grapes. The incidence of gray mold among grape berries that were untreated, or immersed for 1 min in ethanol (35% vol/vol) at 25 or 50°C, was 78.7, 26.2, and 3.4 berries/kg, respectively, after 1 month of storage at 0.5°C and 2 days at 25°C. Heated ethanol was effective up to 24 h after inoculation, but less effective. Ethanol and acetaldehyde contents of grape berries were determined 1, 7, and 14 days after storage at 0.5°C following treatment for 30 or 90 s at 30, 40, or 50°C with water, or 35% ethanol. [Gabler et al., 2005].

In an experiment, grapes have been submitted to three kinds of treatments by dipping in solution of ethanol (50%; 5min), hypochlorite (220ppm of available chlorine; 15min) and hot water (55°C; 5min). Treated and untreated grapes have been stored at $\pm$4°C for 21 days, withdrawn at regular intervals (0, 3, 5, and 7 days. Hot water was able to control gray mold in presence of a small concentration of ethanol. [Karabulut, 2004]. Recently, an ethanol dipping treatment was suggested as a means to prevent Botrytis decay during storage and to extend the shelf life of stored table grapes. Artificially contaminated grapes were exposed to increasing concentrations of ethanol by dipping bunches of grapes for 1–10 min. *E. coli* populations were typically reduced 1–3 log 10 cfu/g on grapes by treatment with 50% ethanol or more, although the results were highly variable. Ethanol treatment, beside its effect on shelf-life extension, can also contribute to minimize *E. coli* populations on grapes and thus enhance their safety. [Pinto et al., 2006]

Ethanol vapor released by the Antimold sachets enhanced berry color, but caused stem browning depending on ethanol vapor concentrations in the headspace of the bags. [Candir et al., 2012].

Conclusion

Under the experimental conditions applied it was demonstrate that the use of ethanol was able to reduce the mold responsible for spoilage without negative manipulation on the product quality.

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