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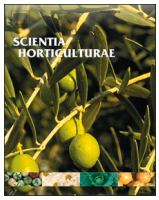

Fresh grapevine (*Vitis vinifera* L.) leaves: Postharvest biology and handling recommendations

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ABSTRACT

Developing grapevine leaves are harvested for the preparation of stuffed dolmas. Fresh leaves are preferred, but since they are perishable and can be harvested at ideal maturity for only a limited period, most leaves for dolmas are currently preserved. This research on fresh grapevine leaves (var. Thompson Seedless) was conducted over three seasons, and focused on their postharvest performance in relation to leaf maturity, respiration rates, storage temperature, water loss and packaging, and decay control. Appropriate leaf maturity for dolmas are leaves that are bright green and not yet fully expanded. The 4 leaf maturity stages that can be harvested commercially for dolmas are clearly delineated by differences in color, and corresponding color values and pigment concentrations. Sugar, acid, and phenolic concentrations and % dry weight vary little among the 4 maturity stages studied. Respiration rates of grapevine leaves (mature for dolmas) are moderate, about 350 μmol CO_2 kg^{-1} h^{-1} at 5 °C. Grapevine leaves can be stored for 4–8 weeks at 0 °C. A storage period of 1 month can be expected if they are stored in the range of 0–5 °C. Grapevine leaves can be stored below 0 °C but freezing injury occurs near -2.5 °C. Water loss at 8–10% is needed to reduce marketable quality (loss of gloss, leaf curling, browning). Botrytis decay is the major limitation to storage life and decay incidence varied from harvest to harvest. Decay is partially retarded by high CO_2 atmospheres (10–15% v/v), but is more effectively controlled by hot water dips of 47.5 °C to 52 °C (15 to 9 min). Grapevine leaves harvested for dolmas do not show yellowing during aging or senescence when held below 10 °C.

1. Introduction

The grape is an ancient crop native to Mediterranean and Central Asian countries, and fresh grapevine leaves are a special byproduct of the vineyards, harvested in the spring from the developing canes before and during flowering. The fresh leaves are used in the traditional cuisines in these areas, and mainly eaten stuffed with meat, rice and vegetables, a dish commonly known as dolma, Turkish for stuffed vegetable (Heine, 2018). While fresh grapevine leaves are preferred, most of the leaves used for dolmas are preserved in brine because the fresh leaves are perishable and because the harvest period of the developing leaves is short, 1–2 months (Sat et al., 2002; Kirca et al., 2006). Good quality fresh leaves should be roundish (14 to 18 cm diameter, $^2/_3$ to ¾ full leaf size), have a fresh appearance, with a typical bright green color and have no decay, discoloration or other defects. As the leaves develop on the vine, they progress from light bright green to bright green to a darker green. The less mature bright green leaves result in the more preferred golden color upon brining, while the darker green leaves develop a brownish color when blanched or brined (Sat et al., 2002; Lima et al., 2016).

Grapevine leaves have a long history as a traditional food and as a folk medicine. Grapevine leaves are considered a healthy food item and more recently have been targeted for potential use in the dietary supplement, pharmaceutical and other industries (Monagas et al., 2006; Katalinic et al., 2013; Pintać et al., 2019). The nutritional and health benefits of the leaves mainly reside in their high concentrations of phenolics, notably flavonoids, flavonols and hydroxycinnamic acid derivatives, which vary considerably among varieties (Orhan et al., 2009; Harb et al., 2015; Lima et al., 2016; Banjanin et al., 2021). Of the grapevine component parts, the leaves, berry skins and seeds have the highest levels of phenolic compounds (Monagas et al., 2006; Šuković et al., 2020). Grapevine leaves have high antioxidant activities (Orhan et al., 2009; Katalinic et al., 2013; Lima et al., 2016; Pintać et al., 2019; Moldovan et al., 2020; Işçimen and Hayta, 2021). Fresh and preserved grapevine leaves are reported to have similar antioxidant activity (Koşar et al., 2007; Juhaimi et al., 2019). Numerous studies have

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described benefits of grape leaf extracts for chronic human health problems (Dani et al., 2010; Fernandes et al., 2013), and antimicrobial and biochemical activities (Fernandes et al., 2013; Katalinic et al., 2013; Altunkaya, 2014). In recent years, there have been many efforts to develop uses for waste and byproducts (grape pomace, seed oil, stems, tendrils, etc.) of vineyards to increase their sustainability and economic competitiveness (Monagas et al., 2006; Spatafora et al., 2013). Fresh grapevine leaves are a product that could attain wider utilization and have higher culinary and economic value than the preserved leaves.

Grapevine leaves are traditionally prepared as a brined product for the dolmas. The brining or pickling conditions can vary considerably, with salt concentrations reported from 4 to 14% alone or in combination with citric or lactic acid and for varying periods of time (Sat et al., 2002; Koşar et al., 2007; Ünver et al., 2007; Gülcü and Demirci, 2011; Lima et al., 2017). The leaves also can be preserved blanched and frozen (Jaradat et al., 2017; Lima et al., 2017), and the leaves can be processed without brine in a traditional fermentation process in hermetically sealed jars at room temperature (Kirca et al., 2006; Koşar et al., 2007). Whatever preservation process is employed, the bright green color of the fresh grapevine leaves should be transformed into the desirable yellow-green olive color, which results from the conversion of chlorophylls to pheophytins and pheophorbides (Kirca et al., 2006; Pareek et al., 2018). Secer et al. (2020) determined that canned stuffed grapevine leaves (dolmas) can have a shelf-life of over 200 days at 25 °C.

There are several companies in California that specialize in the harvest and preparation of grapevine leaves for dolmas. About 90% of the harvested leaves are from Thompson Seedless grapevines, with harvests limited to May and early June when the canes and leaves are developing. The harvesters use a metal piece to cut the grape leaf, leaving no more than 0.5–1.0 cm of stem. Commercial harvests typically result in about 5% of grapevine leaves being picked in a vineyard; a second harvest is done on the same vineyard 3 weeks later (Paul Yergat, personal communication). For the brined product, harvesters pick into a carton box which is then dumped into a plastic field bin lined with a large thick plastic bag (holds about 225 kg) used for the brining process. This amount of fresh product in the field bins can cause heat accumulation due to lack of air movement, resulting in undesirable browning of the fresh leaves before they are brined (Paul Yergat, personal communication). After the proprietary brining process, leaves are often packed into glass jars containing 454 g of grape leaves. The product is brined in bulk in California and then may be shipped to southeast Asia for the laborious selection and packing process. California production of canned grapevine leaves is primarily for the domestic market with some export to the Persian Gulf (Paul Yergat, personal communication). Turkey and Greece export significant amounts of canned grapevine leaves to European and U.S. markets (Secer et al., 2020).

To market fresh grapevine leaves, the expectations of leaf quality are similar to those of the brined leaves in terms of maturity, appearance and freedom from defects. Since the leaves are cooked when used for dolmas, there should be no particular microbial food safety issues, although cleanliness of containers and contact surface should help to minimize microbial contamination and decay. Storage temperatures near 0 °C provide the longest storage life for most temperate leafy greens such as lettuce, kale, spinach (Albornoz and Cantwell, 2016; Cantwell, 2021). It is reasonable to expect that leaves from temperate grapevine varieties would also respond best to low temperature storage. After an extensive literature search, to our knowledge there is no published research (with an abstract in English) on the postharvest biology and handling of fresh grapevine leaves. The research summarized here aims to provide basic information on leaf maturity, respiration, effects of temperature and moisture loss on storage life, and decay control for fresh grapevine leaves. This key postharvest information will help to determine whether fresh grapevine leaves, a more desirable product than brined canned leaves, might be competitively marketed in fresh produce channels.

2. Materials and methods

2.1. Harvest and handling leaves

Leaves were harvested multiple times during May up to early June in each of 3 years (2012, 2013 and 2014) with the help of commercial grapevine leaf harvest crews during early morning hours at Thompson Seedless vineyards in the Fresno area. Leaves were harvested from the middle third of the canes during the shoot growth phase before bloom, stages 15–18 on the Eichhorn and Lorenz growth scale with 8 to 12 leaves per cane (Coombe, 1995; Keller, 2020). The harvested grapevine leaves varied from a light bright green to a dark green in color, with most leaves harvested with a bright green color as preferred for dolmas. For physical and chemical characterization of leaves, 4 stages were harvested in two years, with an estimated 7 to 10 days between the least developed to the most developed stage.

The harvests were from conventional irrigated vineyards that were managed with similar standard practices (Vasquez et al., 2007; UC IPM, 2019). Leaves were cut from shoots using an aluminum cutter fashioned in the shape of a fingernail and taped to the index finger, about 0.5–1 cm from the leaf base. In some experiments, leaves were taken from the harvest containers as these were being dumped into field bins. The harvested leaves were then loosely placed into large plastic bags in large coolers with gel ice packs (wrapped to avoid direct contact with the leaves) for transport to the lab. Leaves typically had a temperature of 18–20 °C when harvested and arrived within 4 h at the lab at 10–15 °C. They were removed from the coolers and the bags (unsealed but turned under to prevent water loss; paper towels added to absorb free moisture) were placed on shelves for cooling and holding at 0 °C until tests were set up.

For the storage experiments, leaves were sorted (removing defective leaves as would be done commercially for the brined leaf product) after harvest and prepared at ambient temperature. Selected leaves were placed on shallow plastic trays (1 tray=1 replicate) covered by unsealed plastic bags (year 1) or placed in clamshells (1 clamshell=1 replicate) enclosed or not in unsealed plastic bags (years 2 and 3). Each replicate contained 12 to 20 leaves (45 to 80 g) depending on the experiment. The multiple-user walk-in controlled temperature rooms were set at intervals of 2.5 °C with Siemens control panels and a fluctuation of ± 0.25 °C. Temperature and relative humidity (RH) were recorded separately in some experiments by Temptale 4 devices (Sensitech, Beverly, MA).

Controlled atmospheres (CA) were prepared by mixing appropriate volumes of humidified air and/or carbon dioxide gas in a flow system at 5 °C. The CO_2 concentrations were measured periodically and were maintained within ± 5% of the stated atmosphere. For the CA tests, product was unwashed, packaged in unsealed polyethylene bags (12 leaves per bag), and placed in polycarbonate containers through which the atmospheres passed at about 5 L/h.

2.2. Quality evaluations

Overall visual quality was evaluated by an experienced operator (first author) on a 9-to-1 scale, where 9 = excellent, fresh appearance, no defects; 7 = good, minor defects; 5 = fair, moderate defects; 3 = poor, major defects; 1 = unusable. A score of 6 was considered the limit of marketability and storage life was defined as the days required to reach a score of 6. Macroscopic decay and damage (due to CO_2 or heat injury) were evaluated on scales of 1 to 5, where 1 = none; 2 = slight (up to 5% surface affected); 3 = moderate (5–20% surface affected); 4 = moderately severe (20–50% surface affected) and 5 = extreme (>50% surface affected). Decay and damage were also calculated as the percentage of leaves with any visible decay or damage. Leaf dehydration was evaluated on a scale of 1 to 5, where 1=none, leaves bright and glossy; 2=slight, leaves less glossy; 3=moderate, leaves with no gloss and slight upturning of leaf edges; 4=moderately severe, leave edges upturned with some browning, and 5=severe, leave edges upturned and brown,

and leaf surface with brown discolored areas. These quality scales are similar to those used for postharvest evaluations of other leafy greens (Albornoz and Cantwell, 2016; Koukounaras et al., 2020).

2.3. Color and nondestructive chlorophyll determination

CIE $L^*a^*b^*$ values were determined on the upper right-hand quadrant of leaves avoiding veins with a Minolta Chroma Meter (Model CR-200/300, Minolta, Ramsey, NJ) with illuminant A and a 10° viewing angle and calibrated on a white reference tile. Chroma ($C^* = [a^{*2} + b^{*2}]^{1/2}$) and hue ($h^\circ = \tan^{-1}[b^*/a^*]$) were calculated (McGuire, 1992). Nondestructive chlorophyll content was determined with a SPAD chlorophyll meter (SPAD 502 DL Plus, Spectrum Technologies, Aurora, IL) on the upper right quadrant of each leaf (Steele et al., 2008).

2.4. Composition

Leaf weight was determined to the nearest 0.1 g. Dry weight (DW) was determined on replicates of freeze-dried leaves, and the resulting dried product was ground into a powder for some composition analyses. Leaf area was calculated from photocopies of leaves which were cut out and weighed, with the relationship 1 g paper = 126 cm^2.

Total chlorophyll and carotenoid concentrations were determined from acetone extracts of fresh tissue from the upper left leaf quadrant frozen at −20 °C until analysis (Lichtenthaler, 1987). A 4 g sample was homogenized (Ultra Max T25 Basic homogenizer, Ika Works, Wilmington NC) for 2 min at 17,500 rpm adding 12 mL acetone 80% (v/v) (10 mg $MgCO_3$ in 1000 mL 80% (v/v) acetone) and then centrifuged (IEC Clinical centrifuge Model CL by International Equipment Company) at 2000 x *g* for 4 min. The supernatant was removed, and the precipitate was extracted once more. The supernatants were combined and volume brought up to 50 ml with acetone, and an aliquot was diluted 10-fold. Total chlorophyll and total carotenoids were measured at 663.2, 646.8 and 470 nm (Shimadzu spectrophotometer UV-1700) and acetone 80% (v/v) (10 mg $MgCO_3$ in 1000 mL 80% (v/v) acetone) was used as a blank. Total carotenoids were determined on the same extract using the extinction coefficient $E_{1\%}$ of 1980 at 470 nm. The contribution of chlorophyll a and b to the measurements was subtracted. Calculations were done according to Lichtenthaler (1987). Chlorophyll calculations: $C_a = 12.25A_{663.2} - 2.79A_{646.8}$; $C_b = 21.50A_{646.8} - 5.10A_{663.2}$. $C_{a+b} = 7.15A_{663.2} + 18.71A_{646.8}$. Total carotenoids: $Car_{total} = (1000A_{470} - 1.82C_a - 85.02C_b)/198$.

Total phenolics were determined on freeze-dried samples by the Folin-Ciocalteu method modified by Singleton and Rossi (1965). A sample of 0.5 g was homogenized (Ultra-Turrax T25, Janke & Kunkel, Staufen, Germany) for 1 min at 13,500 rpm with 20 mL 80% (v/v) ethanol, extracts were centrifuged for 10 min at 24,000 x *g* at 5 °C, and the supernatant was diluted 10 times. Reagent A was 2.7% (w/v) sodium potassium tetrahydrated tartrate, Reagent B was 2.0% (w/v) sodium carbonate in 0.1 N sodium hydroxide, Reagent C was one part of Reagent A plus 98 parts of Reagent B (prepared at time of analysis), and Reagent D was one part of commercial Folin–Ciocalteu reagent and one part of water (prepared at time of analysis). For analysis, 0.25 mL of filtered phenolic extract plus 2.5 mL Reagent C were mixed and left 10 min at ambient temperature. Then 0.25 mL Reagent D was added and tube agitated, and absorbance was measured at 660 nm after 60 min. Calculations were based on a standard curve of *p*-coumaric acid.

Sugar and organic acid determinations were based on methodology described for grapes and grapevine leaves (Hunter et al., 1991, 1994; Lee and Rennaker, 2011). Sugars and acids were determined from a 0.5 g sample of freeze-dried powder thoroughly mixed with 10 mL nanopure water with 3 min of sonication plus 1 min vortexing. Samples were centrifuged at 24,000 x *g* for 10 min at 5 °C, and then passed through a C18 Sep-Pak and a 0.45 μm microfuge spin filter. Sugars were analyzed on an HPLC (Shimadzu system consisting of Shimadzu SIL-1OAD VP autosampler (4 °C), DGU-14A LC-1OAD VP dual-piston pump, and CBM-1OAW VP controller) equipped with a 300 mm x 7.8 mm Aminex HPX-87C column (Bio-Rad, Hercules, CA) and an ELSD detector at 40 °C. The mobile phase was deionized water and the flow rate 0.6 mL min^{-1} with a column temperature of 80 °C. Quantification was done by the use of glucose, fructose, and sucrose standard curves. Organic acids were analyzed using the HPLC with a UV detector at 210 nm and a 300 mm x 7.8 mm Phenomenex Rezex ROA-Organic acid *H*+ (8%) maintained at 40 °C. The mobile phase was 0.01 mol L^{-1} sulfuric acid and the flow rate 0.5 mL min^{-1}. Quantification was done by the use of standard curves generated for tartaric and malic acids. Analyses for both sugar and acids were replicated three times using separate freeze-dried samples.

2.5. Respiration

Respiration rates of grapevine leaves were measured at 0, 5 and 10 °C. About 150 g of fresh leaves were placed in chambers through which humidified air (90–95%) flowed at rates to obtain CO_2 concentrations between 0.25 and 0.5% (v/v) (Kader and Saltveit, 2002). A one milliliter sample was taken from the inlet and outlet streams of the containers, and CO_2 was determined by infrared analysis (model PIR-2000, Horiba, Kyoto, Japan). Calculations were based on a 0.5% (w/w) CO_2 standard and the difference between inlet and outlet concentrations, with respiration rates expressed as μmol CO_2 produced per kg per h.

2.6. Sanitation and hot water treatments

Sodium hypochlorite solutions were prepared with distilled water from 5% (v/v) liquid bleach, and the pH was adjusted to 7.0 with hydrochloric acid (Feliziani et al., 2016). Leaves were exposed to swirling chlorinated water during treatment at 10 °C. After treatment, leaves were removed with a plastic strainer, shaken to eliminate excess water and leaves were separated and placed onto clean cotton towels at 10 °C and blotted dry with other towels to further absorb excess water. After drying, leaves were packaged in clamshells (commercial clamshells for basil) and these were placed on trays enclosed in large unsealed plastic bags and stored at 5 °C on multi-tiered carts. Leaves were evaluated after 12 and 24 days. One replicate consisted of 20 leaves per clamshell. For hot water treatments, 35–40 leaves at a time were swirled manually in a circulating water bath (Isotemp Immersion circulator Model 730) set within ± 0.5 °C of the specified temperature, using 10 L of water for each group of leaves. Treatment temperatures ranged from 47.5 °C to 58 °C (Cantwell and Nie, 1996). After treatment, leaves were blotted dried and prepared for storage as described previously for the sanitation treatments except drying was done at ambient temperature. The hot water was changed after treatment of 3 groups of leaves.

2.7. Experimental design and statistics

Experiments were conducted in a completely randomized design, with a minimum of 3 replicates of 12 to 20 leaves (45 to 80 g) per evaluation, depending on the particular experiment. Leaves were evaluated individually, and details of different experiments are described in the tables and figures. There was one walk-in cooler per storage temperature. Data were analyzed by ANOVA (Sigmaplot 10.0) with treatment and time as main factors. The Fisher Least Significant DPfference (LSD) test was used for mean separation at $P \leq 0.05$ Respiration data were reported as averages ± standard deviations. All experiments were conducted at least twice.

3. Results

3.1. Leaf maturity and composition

Grapevine leaf maturity was evaluated in two years from different

vineyards, with data from year 1 summarized here. Grapevine leaves for dolmas may be harvested at any of the four stages of maturity shown in Fig. 1. While leaves at stage 2 and 3 are the most commonly harvested, the leaves at stages 1 and 2 are preferred, and leaves at stage 4 are considered over-mature for dolmas. Fresh weight per leaf varied from 3.3 to 4.3 g (Table 1). Leaf area increased from 184 cm^2 for stage 1 to 242 cm^2 for stage 4 leaves. Dry weight increased from 24.1% for stage 1 leaves to 25.6% for stage 4 leaves. Total phenolics decreased as the leaves became more mature. Leaf weight, leaf area and % dry weight values were similar in year 2 but total phenolics did not differ significantly among the 4 stages in year 2 (average 57 mg g^{-1}).

Color values, SPAD values and pigment concentrations were different among leaves from each of the 4 stages (Table 2). The CIE hue values and the total chlorophyll concentrations were highly correlated, and each of these in turn were highly correlated to the SPAD values (data not shown). Chlorophyll a/b ratios increased slightly with increasing leaf development, from 1.96 to 2.02 from stage 1 to 4, respectively. Color values and pigment concentrations were very similar between years 1 and 2.

Total sugar concentrations decreased with increasing leaf maturity (Table 3). Both glucose and fructose concentrations decreased with increasing leaf maturity. Glucose, fructose and sucrose averaged 48, 45 and 7% of the total sugars, respectively. Total organic acids were not significantly different among the 4 stages of maturity (Table 3). Tartaric acid comprised 77% of the organic acids on average, with malic acid being the other dominant organic acid. The trends in concentrations of the sugars and acids among the maturity stages were similar between year 1 and year 2. However, total sugars were about 8% lower and total acids were about 5% higher in year 2.

3.2. Respiration

Respiration rates of mature (stages 2 and 3) leaves decreased after harvest (Fig. 2). After 14 days, incipient decay began to appear on the leaves at 10 °C and this was reflected in the higher standard deviations on those days. Over 14 days, respiration rates at 0, 5 and 10 °C averaged 198, 352 and 891 μmol CO_2 kg^{-1} h^{-1}, respectively. In year 2, the average respiration rates of the mature leaves at 0, 5 and 10 °C were 240, 402, and 960 μmol CO_2 kg^{-1} h^{-1}, respectively. In commercial harvests with large quantities of fresh leaves in the bulk bins, some interior leaves can have visible damage (Fig. 5C), likely due to heat generated from high respiration rates and/or oxygen depletion (Kays and Paull, 2004) since the change in leaf color is similar to that observed in a traditional hermetic method of preparation of the leaves for dolmas (Kirca et al., 2006).

3.3. Storage temperature

Stored grapevine leaves showed no visible yellowing, but there was loss of gloss, and curling and browning of leaf margins due to water loss as leaves aged. The main determinant of the potential storage life of grapevine leaves is the development of Botrytis decay (Fig. 3). We considered the end of storage life to be when any symptom of Botrytis was visible, a score of 2. The Botrytis often appeared as 'nesting' infections in the stacked leaves and also often appeared first around the stem end. The relationship between storage life and the development of Botrytis decay is illustrated with the data in Fig. 4. While it is clear that 0 °C provided the longest storage life, the leaves could be stored successfully for 1 month in the range of 0 to 5 °C.

Fig. 1. Four stages of maturity of grapevine leaves. For dolmas, stages 1 and 2 are preferred. However, stages 2 and 3 were the stages most often harvested by commercial crews. Stage 1 leaves are considered immature, stages 2 and 3 are considered mature, and stage 4 leaves are considered over-mature.

Table 1
Fresh weight, area, dry weight, and the concentrations of total phenolics of grapevine leaves harvested at 4 stages of development for dolmas. Data are averages of 3 replicates of 10 leaves each with phenolics determined from freeze-dried tissue. Values within a column followed by different letters are significantly different at $P \leq 0.05$.

Maturity stage	Fresh weight, g per leaf	Leaf area cm^2	Dry weight %	Total phenolics mg *p*-coumaric acid g^{-1}
1	3.28 a	183.5 a	24.1 a	63.5 a
2	3.82 b	216.3 b	25.1 b	53.5 b
3	3.91 b	228.9 bc	25.1 b	52.8 b
4	4.28 c	241.5 c	25.6 b	45.6 c
LSD.05	0.35	18.9	0.7	3.5

Leaves at two stages of maturity were stored at a range of temperatures in one experiment (Table 4; harvest 1 year 1). The storage life of the immature Stage 1 leaves was only slightly less than that of the mature Stage 3 leaves. For example, at 0 °C the immature leaves had a range from 5 to 8 weeks of storage life while storage life of the mature leaves ranged from 6 to 8 weeks. Most of the grapevine leaves harvested for dolmas are at maturity stage 2 or 3 (Fig. 1), considered mature for dolmas, but physiologically immature.

Five storage experiments with freshly harvested grapevine leaves were conducted over 2 years (Table 4). Although there was variation in the results, it is apparent that leaves can be stored for relatively long periods at 0 °C in each of the experiments: for a minimum of 4 weeks to a maximum of 9 weeks. In two tests, the leaves were stored at −2.5 °C and although they could be stored longer at this temperature than at 0 °C, they eventually developed freeze damage (Fig. 5B) due to sporadic temperature fluctuations in the room. It would be reasonable to expect that the leaves could safely be held at −2 to −1 °C without freeze damage.

3.4. Water loss

Grapevine leaves can tolerate relatively high levels of water loss. They can lose up to about 5% fresh weight without affecting gloss, and lose up to about 10% fresh weight without affecting overall marketable quality or objective color values (data not shown). Fig. 6 shows the time course of water loss at 4 temperatures with grape leaves packaged in vented clamshells with or without additional plastic protection (enclosed in unsealed bag). The relative humidity inside the unprotected clamshells was 90 to 92% across the 4 temperatures, while the RH inside the protected clamshells was 94 to 96%. The main difference in weight loss was due to airflow around the unprotected clamshells. After 5 weeks, the leaves in protected clamshells at 0 °C retained excellent quality with no dehydration and very little decay. The leaves in unprotected clamshells at 0 °C were of marketable quality although they had some dehydration (slight curling of the leaf margins) and very little decay. Leaves stored at warmer temperatures had more dehydration in the unprotected clamshells and more decay in the protected clamshells. Another packaging test was conducted in year 3 at 0, 2.5 and 5 °C. In this test, the leaves stored in bulk at 0 °C for 6 weeks, were selected for quality and packaged, and then held for an additional 3.5 weeks at 5 °C (to simulate commercial storage and a retail marketing period). Results were similar to those in Fig. 6 except that decay incidence was higher in all treatments and there were few differences in dehydration symptoms.

Table 2
CIE color values, SPAD values, and pigment concentrations of grapevine leaves harvested at 4 stages of development for dolmas. Data are averages of 3 replicates of fresh tissue from 10 leaves each. Values within a column followed by different letters are significantly different at $P \leq 0.05$.

Maturity Stage	L*	a*	b*	Chroma	Hue	SPAD values	Total chlorophyll mg g^{-1}	Total carotenoids mg g^{-1}
1	43.9 a	−17.0 a	25.6 a	30.7 a	123.6 a	22.6 a	0.83 a	0.14 a
2	40.8 b	−16.2 b	22.3 b	27.6 b	126.3 b	26.9 b	0.97 b	0.17 b
3	38.7 c	−13.8 c	16.4 c	21.4 c	130.2 c	30.7 c	1.20 c	0.20 c
4	36.3 d	−11.6 d	12.4 d	17.0 d	133.1 d	34.3 d	1.37 d	0.22 c
LSD.05	1.4	0.8	1.8	1.9	1.4	0.7	0.12	0.02

Table 3
Sugar and organic acid concentrations in grapevine leaves at 4 stages of development for dolmas. Data are averages from freeze-dried tissues from 3 replicates of 10 leaves each. Values within a column followed by different letters are significantly different at $P \leq 0.05$.

Maturity Stage	Sugars, mg g^{-1}				Organic acids, mg g^{-1}		
	Glucose	Fructose	Sucrose	Total	Tartaric	Malic	Total
1	87.2 a	71.5	12.2	170.9 a	68.6	15.3	83.9
2	67.1 b	67.9	8.0	143.0 b	74.9	19.7	94.6
3	66.4 b	61.4	9.5	137.2 bc	70.3	22.3	92.6
4	60.9 b	58.9	12.8	132.6 c	60.5	26.4	86.9
LSD.05	10.4	ns	ns	7.2	ns	ns	ns

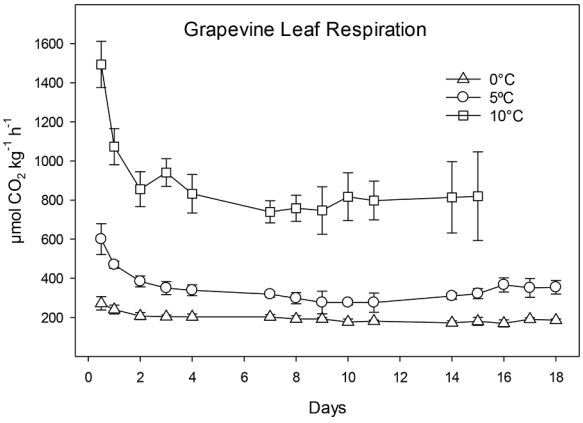

Fig. 2. Respiration rates of mature (stages 2 and 3) grapevine leaves. Data are averages of 3 replicates of 20 leaves each ± standard deviation.

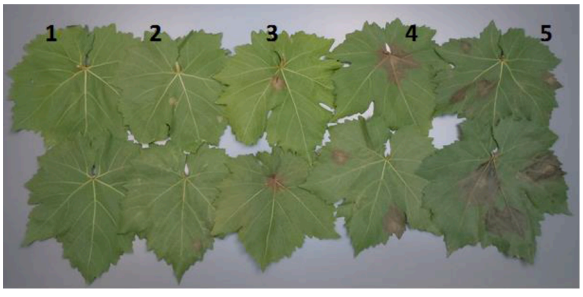

Fig. 3. Botrytis decay on the abaxial side of grapevine leaves, showing the rating scale used, where 1=none, 2=slight, 3=moderate, 4=moderately severe and 5=severe. A score of 2 corresponds to the end of storage life.

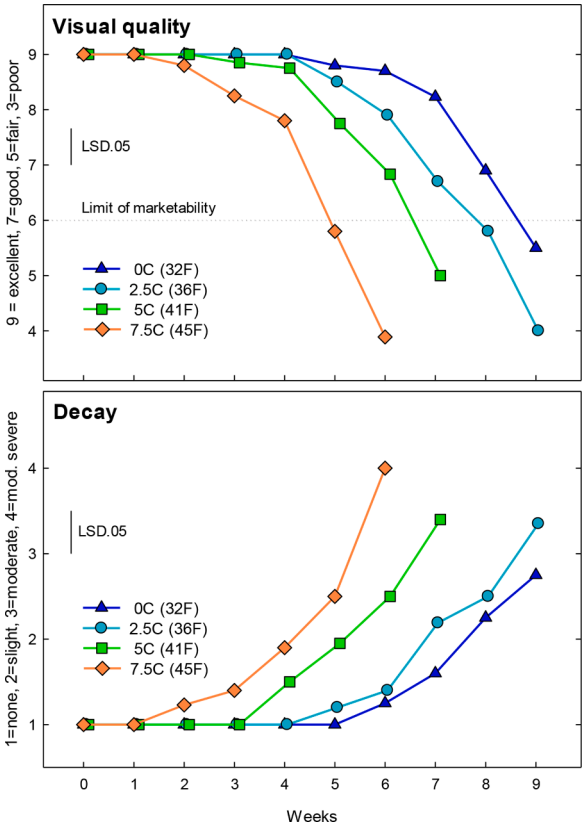

Fig. 4. Quality changes of mature (stage 3) grapevine leaves stored at 4 temperatures. Data are averages of 4 replicates with 20 leaves each (one clamshell) per evaluation. Visual quality was scored on individual leaves on a scale of 9=excellent to 1=unusable, with a score of 6 being the minimum for marketability. Decay was scored on individual leaves on a scale of 1=none to 5=severe.

3.5. Decay control

3.5.1. Controlled atmospheres with high CO_2

Since high CO_2 concentrations are effective to control Botrytis in products such as grapes and berries, two experiments were conducted with high CO_2 atmospheres. An atmosphere containing 15% (v/v) CO_2 was effective in retarding decay for 4 weeks at 5 °C (Table 5). An atmosphere containing 7.5% (v/v) CO_2 was ineffective after 4 weeks, with decay incidence similar to that observed on air-stored leaves, but the 15% CO_2 provided excellent decay control After 6 weeks, however, the 15% CO_2 atmosphere provided only modest benefit. The 15% CO_2 atmosphere caused a slight amount of injury at 4 weeks, and notable injury at 6 weeks (Fig. 5A). These experiments were done at a storage temperature of 5 °C, which is relatively high but would be a typical temperature during distribution and marketing. In a second experiment

Table 4
Weeks of storage life of unwashed grapevine leaves from different experiments. In year 1, leaves were stored in plastic bags; in year 2, leaves were stored in clamshells covered by plastic bags. Storage life ended when there was any visible decay. See Fig. 1 for leaf maturity stages and Fig. 2 for decay scale.

		Storage temperature, °C						
Harvest	Maturity	−2.5	0	2.5	5	7.5	10	15
Harvest 1 year 1	Immature		5–8		3–5		2–3	1
Harvest 1 year 1	Mature		6–8		4–6		2–3	1–2
Harvest 2 year 1	Mature		4–9		3–6			
Harvest 3 year 1	Mature	9–10*	6–9		4–6		3	
Harvest 1 year 2	Mature	10–12 *	7–8	6–7	4–6	3–4		

* Estimates as many leaves had freezing injury after 8 weeks (year 1) and 9 weeks (year 2) due to sporadic temperature fluctuations below −2.5 °C.

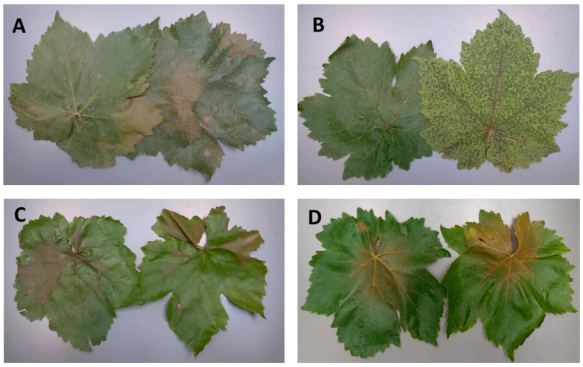

Fig. 5. Grapevine leaf defects: high CO_2 injury (A), freeze damage on upper and lower sides (B), injury due to high heat of respiration or oxygen depletion in leaves from commercial harvest bins (C), and hot water injury (D).

with 10 and 15% CO_2 atmospheres, 10% CO_2 provided less control than 15% CO_2 with no injury to the leaves, and again the 15% CO_2 provided good Botrytis control for up to 4 weeks at 5 °C (data not shown). The 15% CO_2 atmosphere caused no injury to the leaves after 4 weeks, but did cause injury after 6 weeks, while the 10% CO_2 atmosphere did not cause any visible injury at 6 weeks.

3.5.2. *Washing with chlorinated water*

Fresh grapevine leaves may have sulfur spray residues when harvested and post-storage washing would be a possible handling step. One experiment was conducted evaluating the efficacy of sodium hypochlorite in the wash water to retard decay growth. Freshly harvested leaves were washed in swirling water, or in 150 or 300 mg L^{-1} NaOCl at 10 °C for 5 min. The leaves were drained and dried and then stored in protected clamshells for up to 38 days at 5 °C. At 30 days, unwashed control leaves had no decay, but with an additional 8 days, 32% of the leaves had some decay with an average score of 1.8 (slight). For the water-wash treatment at 38 days, 86% of the leaves had some decay with an average decay score of 3.4 (above moderate). At the same time, the chlorine-containing wash waters resulted in less decay than for the water wash alone, with 58% and 65% leaves with decay in the 150 and 300 mg L^{-1} NaOCl treatments, respectively, and decay scores were 2.5. Washing the grapevine leaves increased the incidence of Botrytis decay in the stored product, however, washing the fresh leaves may be needed to remove dust and sulfur spray residues for fresh market channels.

3.5.3. *Post-storage decay control for retail marketing*

Decay development on the grapevine leaves can vary from one harvest to another. For commercial handling, it is assumed that leaves would be stored in bulk and then prepared for market. Post-storage decay control is therefore needed, and research here focused on hot water dips, alone or in combination with NaOCl. These five experiments were conducted in year 3. Bulk leaves were stored at 0 °C for 6 weeks, and then leaves without any visible decay symptoms were selected for treatment and packaging. Hot water treatments were applied in the range of 47.5 to 58 °C, with corresponding exposure times of 15 to 1 min (Fig. 7). About a 2% weight increase was observed after dipping at 49 °C for 10 min. The shorter time, higher temperature treatments were less effective and usually caused some injury (Fig. 5D), while treatments at temperatures of 47.5 to 52 °C were effective and caused no injury. Some of the treatments included 150 mg L^{-1} NaOCl. The best hot water options for decay control are shown in Fig. 8. Both the 47.5 °C water for 15 min and the 50 °C water for 9 min provided excellent decay control. The addition of NaOCl to the hot water dip did not further improve decay control. These two hot water treatments did not damage the leaves and were effective for a marketing period of 14 to 21 days at 5 °C.

4. Discussion

Developing leaves from Thompson Seedless grapes are harvested in the spring up to flowering for use in the preparation of dolmas. The grapevine leaves are harvested at different stages of maturity that are easily distinguishable in the field and which have significant differences in color values and concentrations of chlorophyll and carotenoids. Both hue color values and the SPAD values are useful for quality control since these measurements are easy to perform nondestructively on the fresh leaves. Majer et al. (2010) found that hue and SPAD measurements were comparable for estimating chlorophyll concentrations and photosynthetic activity in grapevine leaves. However, Steele et al. (2008) concluded that a calculated chlorophyll index derived from $L^*a^*b^*$ color values was a more accurate assessment of chlorophyll concentration than SPAD measurements. As grapevine leaves mature they progress from light bright green to a darker green; the lighter green leaves result in a more golden color upon brining, while the darker green leaves result in a less desirable brownish color when preserved (Kirca et al., 2006). Most of the grapevine leaves are harvested at the bright green stage (stages 2 and 3, Fig. 1) and are considered mature for use in dolmas. The leaves at these stages are still physiologically immature and contain less chlorophyll and have less photosynthetic activity than fully mature leaves (Filimon et al., 2016). Typical leaves harvested for dolmas have 70–85% of their maximum chlorophyll content, which occurs between fruit set and veraison (Bertamini and Nedunchezhian, 2003; Filimon et al., 2016). About 5% of the leaves in a vineyard are harvested on two occasions for use in dolmas and this small percentage of leaf removal has no repercussion on grape yield or quality (Palliotti et al., 2011).

The four stages of maturity of grapevine leaves appropriate for harvest for dolmas have some differences in composition in addition to the notable differences in color and pigments. Total sugars and phenolics decreased over the 4 stages while there were no significant differences in organic acids. The percent dry weight increased only 1.5% over the 4 stages. Other research has described greater changes in sugars, acids, individual phenolics and other bioactive compounds in relation to grapevine leaf maturity (Kliewer and Nassar, 1966; Gülcü et al., 2020). In these reports, however, young to physiologically mature leaves were analyzed, a much longer developmental period than considered in the present study.

The only data on respiration rates of grapevine leaves are those of Zufferey (2016) on a per area basis and those of Hernández-Montes et al. (2019) in which dark respiration was measured *in situ* at ambient temperatures. The latter results are comparable to the results presented here. The respiration rates of fresh grapevine leaves are similar to those of romaine lettuce (Cantwell and Suslow, 2001) and salad-cut kale (Albornoz and Cantwell, 2016), and are considered moderate rates for leafy greens. In the field bins, the heat from respiratory metabolism may

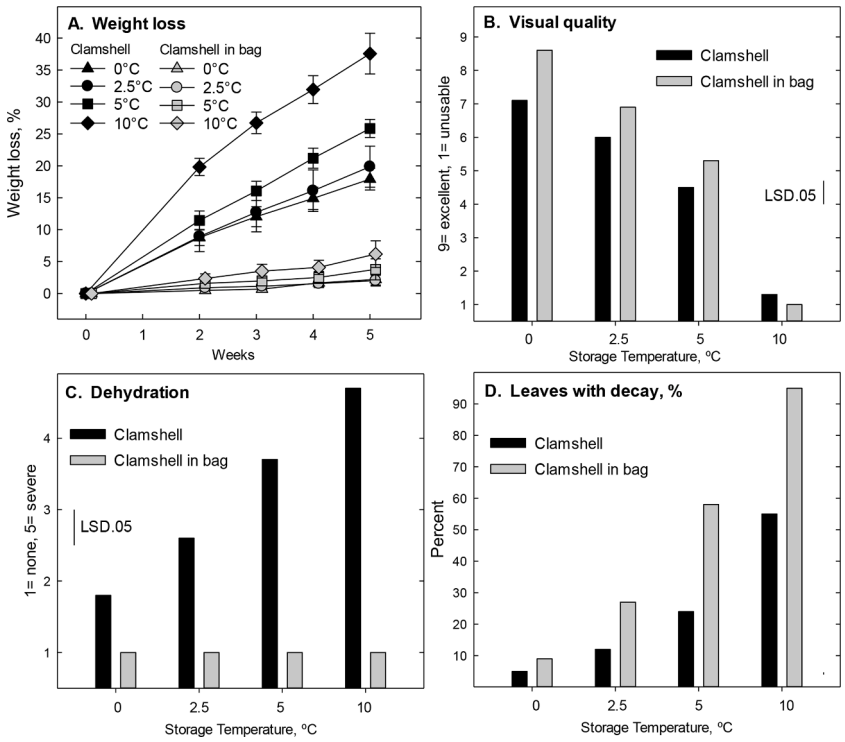

Fig. 6. Grapes leaves packaged in clamshells with and without a plastic bag cover were stored at 4 temperatures: Weight loss during 5 weeks (A), and visual quality (B), dehydration (C) and leaves with any visible decay (D) at 5 weeks. Quality data are averages of 6 replicates of 20 leaves each per treatment, scoring leaves individually.

Table 5
Percent of leaves with incipient visible decay, and decay and discoloration scores for grapevine leaves stored at 5 °C for 4 and 6 weeks in air, or in air plus 7.5% or 15% (v/v) CO_2. Data are averages from 12 bags (replicates) of 12 leaves each. Decay and discoloration were scored on 1 to 5 scales, where 1=none, 2=slight, 3=moderate, 4=moderately severe and 5=severe. Values within a column followed by different letters are significantly different at $P \leq 0.05$.

Treatment	4 weeks 5 °C Leaves with visible decay,%	Decay score	Discoloration score	6 weeks 5 °C Leaves with visible decay,%	Decay score	Discoloration score
Air	32	1.6 a	1.0 a	82	3.4 a	1.0 a
7.5% CO_2	31	1.7 a	1.0 a	90	3.3 a	1.0 a
15% CO_2	6	1.1 b	1.2 b	65	2.2 b	2.9 b
LSD.05	–	0.3	0.2	–	0.3	0.2

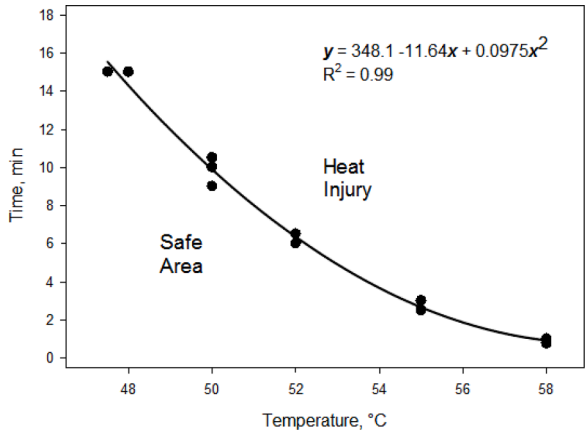

Fig. 7. The time-temperature relationship for non-injurious hot water treatment of grapevine leaves to control Botrytis decay. Treatments at temperatures above 52 °C were less effective for decay control than hot water treatments in the range of 47–52 °C.

accumulate if there is a lack of sufficient air flow (Kays and Paull, 2004), and this may lead to undesirable browning of the leaves (Paul Yergat, personal communication).

Grapevine leaves have a very high% DW compared to other leafy greens. Dry weight of mature grapevine leaves averaged 24% in year 1 and 2, and these values are similar to others reported for fresh grapevine leaves (Sat et al., 2002). Lettuces may have 5–8% dry weight, kales range from 9 to 14% dry weight, red and green cabbages range from 9 to 18%, and arugula may vary from 8 to 14% dry weight (Cantwell, unpublished). A higher dry weight in leafy greens is associated with longer storage life (Conte et al., 2008; Cantwell, unpublished). The high dry weight of the grapevine leaves may contribute to the fact that they have a relatively long storage life (weeks at 0 °C).

The high dry weight or solids content of the grapevine leaves also contributes to their ability to be stored below 0 °C. Storage below 0 °C, referred to as near freezing point storage (Liu et al., 2019) or supercooling (Quang et al., 2017), is of increasing interest for storage of a wide range of perishables (Stonehouse and Evans, 2015). In California, garlic and plums are often stored at −2 °C because of their low freezing point due to high solids content. The recommended storage temperature for grape berries is −1 °C (Chervin et al., 2012). In the present study, grapevine leaves retained very good quality at −2.5 °C although freeze damage eventually occurred due to unfortunate sporadic temperature

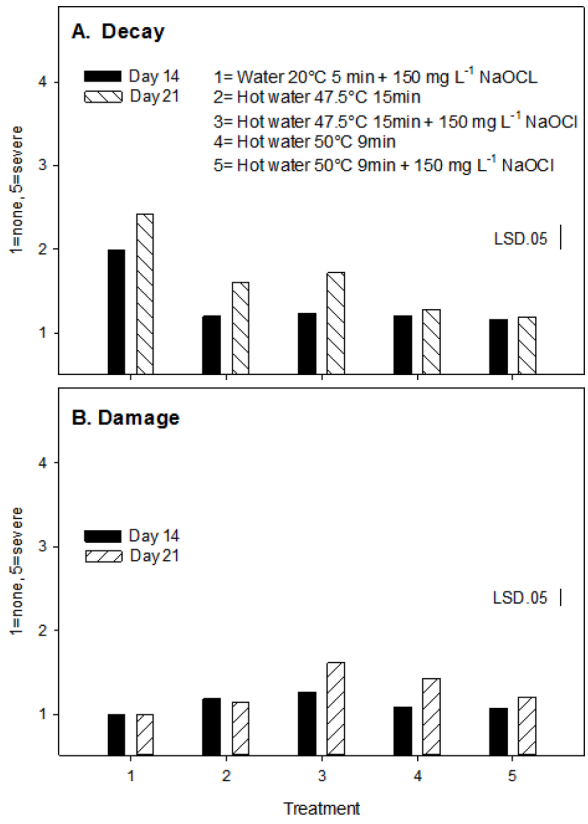

Fig. 8. Effective hot water treatments alone or in combination with sodium hypochlorite to control Botrytis decay on grapevine leaves. Leaves were stored for 6 weeks at 0 °C prior to treatment to simulate bulk commercial storage. The treated leaves were then stored in clamshells in unsealed plastic bags at 5 °C for 2 and 3 weeks to simulate commercial marketing. Data are averages from 4 replicates of 15 leaves each per treatment per evaluation.

fluctuations below the setpoint. A temperature of −3.5 °C was used to study freezing injury damage on grapevine leaves and shoots (Centinari et al., 2016). Storing fresh grapevine leaves at −2 to −1 °C should significantly extend their storage life beyond what can be achieved at 0 °C (Stonehouse and Evans, 2015). The technical expertise and infrastructure to store near freezing point is already available in many grape growing areas (Chervin et al., 2012).

Within the timeframe and experimental conditions of this study, yellowing, a manifestation of leaf senescence, was not observed. Perhaps more abusive storage conditions, such as higher temperature (>10 °C) and longer storage (>38 days) would eventually lead to some chlorophyll *b*reakdown, although the immature leaves may age without this typical sign of senescence. Since the grapevine leaves were not fully mature and had not reached their peak chlorophyll content at the moment of harvest (Filimon et al., 2016), the senescence mechanisms were probably not in place, thus allowing a prolonged chlorophyll retention during storage. In other leafy greens, kale (Albornoz and Cantwell, 2016) and lettuce (Witkowska and Woltering, 2014), younger leaves performed better than older leaves in storage, possibly because mechanisms underlying senescence, such as decrease in photosynthetic activity, loss of cellular integrity and lipid degradation were already operating in the older tissues before the onset of visible symptoms like leaf yellowing (Yoshida, 2003; Buchanan-Wollaston et al., 2005; Noodén, 2013; Ruberti et al., 2014). Yellowing of fully mature detached grapevine leaves did occur at warm temperature (28 °C) (Shi et al., 2019).

The main symptoms of deterioration, other than decay, are curling of the leaf edges due to water loss with subsequent browning of the leave edges and the leaf surface. However, Botrytis decay is the main mode of storage failure in grapevine leaves protected from water loss. In some experiments, nesting of the Botrytis decay was observed. The time course of storage life and decay development are almost mirror images of each other. Most stored leaves will eventually develop Botrytis decay and if the leaves are stored in bulk they must be monitored so that they can be marketed before decay becomes an issue. While effective for other products (Feliziani et al., 2016), chlorine water washes at ambient temperature were ineffective to reduce decay on grapevine leaves. Instead, washing increased decay even when leaves were well-dried before storage, and chlorine in the wash water did little to reduce the increased decay. In this study, we focused on the use of post-storage hot water dips, previously demonstrated to be very effective to control Botrytis decay (Cantwell and Nie, 1996; Sui et al., 2016). Hot water treatments at 47.5 °C for 15 min or 52 °C for 9 min were particularly useful in retarding decay growth on leaves that had been stored in bulk for 6 weeks, among which there were leaves with visible decay symptoms. Leaves with no visible decay were selected, treated and packaged for a simulated distribution period of 3 weeks. For the grapevine leaves, such a batch type treatment, either hot water or hot forced air (Cantwell and Nie, 1996), would likely be feasible commercially. Heat treatments may have other benefits besides decay control such as reduced yellowing in leafy greens (Fallik and Ilić, 2020) and other physiological changes that extend storage life (Lurie, 2018). However, since yellowing does not occur in the grapevine leaves stored below 10 °C, the main benefit of the heat treatment is Botrytis control.

There has been extensive research conducted on Botrytis decay control on grape berries (Chervin et al., 2012; Feliziani et al., 2016), and likely some of the treatments used on grapes could be effective on grapevine leaves. High CO_2 atmospheres retarded Botrytis decay in Thompson Seedless grapes (Retamales et al., 2003). In this study, grapevine leaves easily tolerated 10% CO_2 and were injured only slightly with 15% CO_2, but the treatments were not very effective to control Botrytis decay. The pre-storage SO_2 fumigations and SO_2 pads used in packed grapes for Botrytis control may also be tolerated by the leaves. Since the leaves are cooked, concerns over sulfite residues would not be an issue (Chervin et al., 2012). Ozone may be a possible treatment as it can be applied as a gas at low continuous doses in storage to prevent the germination and development of fungal spores (Smilanick et al., 2012; Glowacz et al., 2015; Shezi et al., 2020).

It would be important to assess the impact of proposed storage conditions on the sensory quality of the prepared dolmas. For example, the color of the prepared dolma may not be the same with increased storage time of the fresh grapevine leaves. Also, it could be expected that the flavor sensory quality of the prepared dolma could change with storage time of the fresh leaves. Such evaluations should be a focus of future postharvest work on fresh grapevine leaves. For the brined dolmas, sensory quality declines significantly over a 4-month period (Ünver et al., 2007).

Our research to date allows us to propose a likely handling scenario for fresh grapevine leaves. Harvested leaves (maturity stage 2 and 3) would have a fresh appearance with typical bright green color and freedom from defects. The harvest tool (metal fingernail), containers and bins would be used only for the fresh market product and have improved sanitation to help reduce Botrytis decay. Leaves would be harvested into plastic totes or half-bins, transported rapidly from the field, forced-air cooled within 2 h, and stored at −1 °C for up to 2 months. The needed infrastructure and technology exist already in grape growing areas. Possibly periodic SO_2 fumigation or continuous low dose ozone could be used in the storage room to retard decay growth. Different lots would be monitored for the first signs of Botrytis decay so they could be marketed expeditiously. For that, bins and/or totes would be brought out to a refrigerated (<10 °C) packing area for selection, detail work (possible wash step; possible hot water treatment) and packaging. The product could be packaged in clamshells with additional protection to reduce water loss. Alternatively, the leaves could be placed in modified atmosphere packaging (MAP) to provide a high CO_2

atmosphere to reduce decay. Additional decay control measures may be needed. Transportation and distribution should be done at 0–2.5 °C to minimize decay and to achieve a post-storage shelf-life of 14 to 21 days. Since the product is eaten cooked, there would appear to be no particular microbial food safety issues.

5. Conclusions

This study provides basic postharvest information about fresh developing grapevine leaves harvested for use in dolmas. Respiration rates of bright green grapevine leaves are moderate compared to other leafy green vegetables. Grapevine leaves can best be stored at 0 °C or lower near the freezing point. The storage life is limited by the development of Botrytis decay, whose incidence varies from harvest to harvest. Grapevine leaves tolerate up to about 8–10% weight loss before there is a significant impact on marketability. Grapevine leaves tolerate high levels of CO_2 (10%) and therefore could benefit with packaging in a microperforated MAP. Grapevine leaves tolerate hot water treatments which are very effective to control Botrytis decay. More work is needed on decay control options, and handling logistics to develop a commercial postharvest value chain for fresh grapevine leaves. The impact of storage and handling conditions of the fresh leaves on the final sensory quality of the prepared leaves should also be evaluated.

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Author statement

The studies were planned by Cantwell and work was executed by all authors. The data were analyzed by Cantwell and Hong. The first draft was written by Cantwell and revised by all authors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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