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Dangler for Accelerated Dehydration: A Novel System for Assessing the Impacts of Relative Humidity on Fruit Water Loss During Cold Storage of Blueberries

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Abstract. **Blueberries are prone to dehydration during storage. Firmness is one of the most critical quality attributes associated with this period, with the loss of water from the fruit representing the most significant limitation for the fresh market. Therefore, one of the great challenges is maintaining the quality characteristics of the fruit in shipments by sea, which can take up to 60 days when sent from the southern hemisphere to the northern hemisphere. The random arrangement of each fruit within a packaging unit (different proportions of the stem scar and cuticular surface exposed to the environment) represents an essential source of variation in the prediction of softening during the storage period. A special device, referred to as a dangler for accelerated dehydration (DAD), was designed to expose nearly the entire fruit surface to the environment and determine the impact of factors such as relative humidity and the role of the stem scar and cuticle on fruit water loss. Consequently, to evaluate the ability of DADs to find differences in fruit dehydration, blueberries sampled at early, peak, and late harvest dates were placed in DADs and exposed to three controlled levels of relative humidity (30%, 65%, and 96% relative humidity; 1.2 ± 0.7 °C) for 10 days. Berries within the DADs were untreated, immersed in hexane for 5 seconds to remove bloom, painted with quick-drying nail polish on the pedicel end to seal the stem scar or immersed in hexane for 5 seconds, and painted with quick-drying nail polish on the pedicel end. At each harvest, fruit weight loss was significantly affected by the fruit and RH treatments, as well as the interaction between them. A regression analysis of the control treatment indicated that water loss at lower relative humidities occurred faster in fruit from the first harvest. The results reveal that DADs can be used to characterize preharvest and postharvest stimuli at an individual level and within a short time (10 days).**

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Fresh blueberries are prone to dehydration during postharvest storage and transport, thus limiting their fruit quality when reaching final markets (Rivera et al. 2022a). Regarding fruit quality traits, firmness is the most relevant attribute when blueberries arrive at destination markets (Chiabrando et al. 2009; Rivera et al. 2022b). Therefore, postharvest softening is the main obstacle for long-term shipments (Giongo et al. 2013; Vicente et al. 2007). Softening of blueberries during storage is closely associated with moisture loss (Paniagua et al. 2013, 2014), with significant genotypic dissimilarities between seasons, especially during long-term storage (Alsmairat et al. 2011; Moggia et al. 2017a; Paniagua et al. 2013; Sargent et al. 2006; Vicente et al. 2007).

Although blueberry breeders have released genotypes with improved firmness potential (Cappai et al. 2018), environmental conditions such as temperature and rainfall modulate their expression, leading to genotype × environment interactions (Almutairi et al. 2021; Estrada et al. 2015; Lobos et al. 2012, 2013; Moggia et al. 2014; Moon et al. 1987a, 1987b; Spann et al. 2004; Tasnim et al. 2021; Yáñez et al. 2005). Management practices such as the timing, frequency of fruit harvest, and handling are important to maintaining fruit quality from the field to consumption (Forney 2009; Ktenioudaki et al. 2021), particularly for long-term shipments, which normally require up to 60 d; however, after the COVID-19 pandemic, this has been extended by up to 20 d.

Air temperature and relative humidity (RH) management are critical to minimizing the postharvest dehydration and consequently softening of the fruit. Both are used to calculate the vapor pressure deficit, which is directly related to dehydration and softening (Whitelock et al. 1994). The water content of fresh blueberries is normally approximately 84% (Lobos et al. 2013), and the best conditions for storing blueberries are 0 °C and RH >95% (Forney 2009).

Among the main pathways of water loss, both the stem scar and the cuticle have been proposed as the most relevant barriers influencing postharvest dehydration in blueberries (Lara et al. 2014; Moggia and Lobos 2023; Moggia et al. 2016, 2017b, 2018; Shepherd and Wynne 2006; Wang et al. 2022; Yan and Castellarin 2022). Although the area of the stem scar corresponds to <1% of the fruit surface, it is responsible for 40% to 60% of water loss (Moggia et al. 2017b). Therefore, the cuticle is an essential constituent of the stabilizing structure of the epidermal tissues and modulates water permeability during postharvest life (Lara et al. 2014; Qi et al. 2019; Shepherd and Wynne 2006).

Firmness at harvest is not only a function of genotype×environment×management but also a function of the conditions influencing growth and development up to the time of the picking, which is particularly relevant for long-distance shipments. A harvest index used by the blueberry industry (100% blue cover) that prevents the picker from discriminating between ripe and overripe berries and random spatial arrangement of each fruit within the clamshell packaging units (different proportions of stem scars and cuticular surfaces exchanging gases with the environment) represent other important sources of dehydration and fruit softening variations within the same lot.

During different seasons, fruit harvested from the same orchard and cultivar may have the same initial firmness but display different rates of softening during postharvest, thus explaining the lack of association reported between firmness measured at harvest and firmness at the end of the storage period (Lobos et al. 2018; Moggia et al. 2022). Therefore, the study of dehydration at the individual fruit level seems to be a more objective alternative to determine the impact of a stimulus on the postharvest of the fruit. This information is relevant to the scientific community but could also represent a practical

and easy-to-implement methodology for the blueberry industry.

Consequently, this work aims to demonstrate that studying daily dehydration at the individual fruit level using a novel Dangler for Accelerated Dehydration (DAD) three-dimensional (3D) printing device that allows the fruit to be almost completely exposed to the environment is an effective and simple way to characterize the impact of any stimulus (e.g., harvest time). Because the potential for fruit water loss is most likely related to the conditions or stimuli under which the scar and cuticle are developed during a particular season, we hypothesized that when the fruit surface is mostly exposed to an RH gradient (i.e., 30%, 65%, or 96%), the rates of weight loss through the pedicel scar and cuticle should vary as a function of the stimulus being evaluated.

Materials and Methods

The trial was conducted in an 8-year-old field of 'Brigitta' northern highbush blueberry (*Vaccinium corymbosum*) located at a commercial farm in Linares, Maule Region, Chile (35°49′43.4″S, 71°33′37.4″W) during the 2020 to 2021 growing season.

Approximately 500 berries (100% blue) were sampled from the northwest side of the plants at a height of 1.5 m on three harvest dates during the season: early (26 Dec 2021), peak (5 Jan 2022), and late (15 Jan 2022) commercial picking. The fruit was placed in clamshells and immediately transported to the postharvest laboratory of the Plant Breeding and Phenomics Center, Universidad de Talca, Talca, Chile.

To study postharvest dehydration at the individual fruit level, humidified air-flow units were constructed to expose the berries to three levels of RH for 10 d (Fig. 1A). The system, which is based on the design by Forney and Brandl (1992) and Paniagua et al. (2013), was installed inside a conventional cold chamber (1.2 ± 0.7 °C and 95 ± 0.3% RH). Briefly, air (5.5 L·min^{-1}; SB-9905A, Sobo, India) was pumped through plastic hoses (4-mm diameter) into solutions containing different concentrations of glycerol (99.95%) to generate RH levels of 96% (100% bidistilled water), 65% (75% glycerol and 25% bidistilled water, by volume), and 30% (100% glycerol) (Supplementary Fig. S1). Each RH level was replicated three times using independent air pumps and solutions for the chamber. Thereafter, the air flowed into transparent PVC containers (35 × 24 × 14 cm; 11.7 L) with fruit samples. A special device, referred to as a DAD, was designed to support the fruit in the containers and expose nearly the entire surface of the berries to the environment during cold storage (Fig. 1B). The DADs were 3D-printed (PETG; 6 g) and consisted of two assembly parts, the body (parallelepiped: 86 × 63 × 2 mm) and the baskets (truncated cone: 21 × 17 mm diameter × 12 mm), which can hold five berries at the same time. The "*.stl" files of the 3D-printed material are included as supplementary information (Supplementary Materials S1, S2).

To avoid possible position effects caused by air flow (i.e., greater RH toward the air

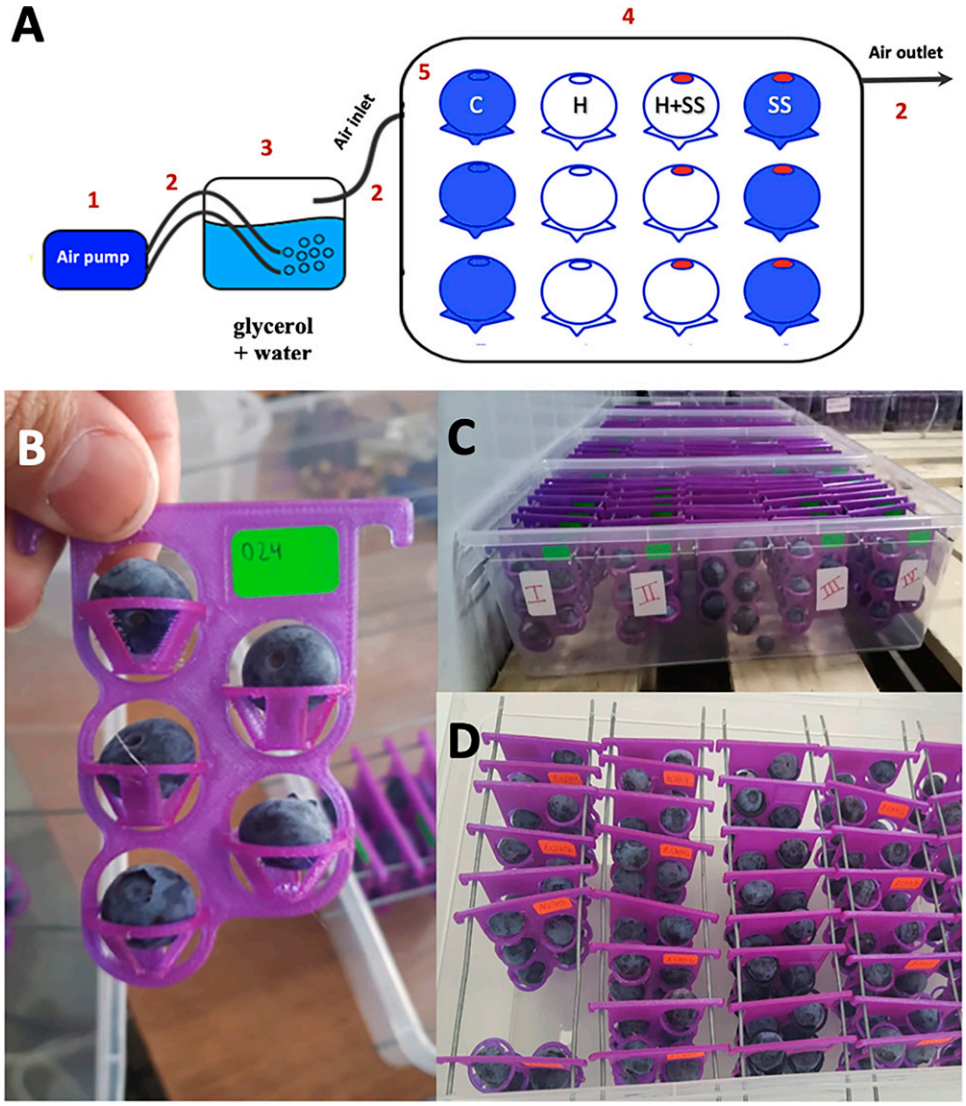

Fig. 1. (**A**) Humidified air-flow system for controlling relative humidity during cold storage of blueberries: air is pumped (**1**) through plastic hoses (**2**) into a container filled with a mixture of water and glycerol (**3**). Thereafter, air flows into an adjacent sealed container (**4**) with fruit samples (**5**). (**B**) A special device referred to as a dangler for accelerated dehydration (DAD) was designed to hold the fruit samples. The DADs were arranged in four blocks in each container (**C**) and supported by wire rails (**D**). A buffer row of DADs was included in the center of each box.

outlet of the box), each PVC container was divided into four blocks (Fig. 1C). A buffer block was also included in the center of each container, where temperature and RH sensors were placed. In each block, eight DADs (i.e., two DADs per treatment and block) were supported by two wire rails (2-mm diameter) located across the container (Fig. 1D).

To promote rapid cooling, berries were immediately placed in the DADs, and the boxes were kept open for 24 h in the cold room. The next day, each DAD (five berries) was weighed, the containers were sealed, and the RH generation systems were activated. Using a high analytic scale (M214AI; MEL, Monza, Italy), weight loss of each DAD was recorded daily at 12:00 PM for 10 d inside the cold chamber (1.2 ± 0.7 °C) to avoid temperature changes.

Temperature and RH were recorded every 5 min using automatic sensor units (HOBO U23 Pro v2 Temperature/Relative Humidity; Onset Computer Corporation, Bourne, MA, USA).

To assess the sensitivity of this new system, the following treatments were applied to DADs: untreated berries (control); berries immersed in hexane for 5 s to remove the bloom (hexane treatment) (Post-Beittenmiller 1996); berries with the stem scar sealed with quick-drying nail polish (stem scar treatment) (Ultimate Nail Lacquer; Catrice Cosmetics, Sulzbach, MTK, Germany) (Moggia et al. 2017b); and berries treated with a combination of bloom removal and sealed stem scars (hexane plus stem scar treatment). A randomized complete block design with a 3 (RH) × 4 (fruit treatments) factorial arrangement was used on each of the three picking dates. At each harvest (i.e., early, peak, and late), the combined effect of RH (i.e., 96%, 65%, and 30%) and fruit handling (i.e., control, hexane treatment, stem scar treatment, and hexane plus stem scar treatment) on water loss (g) was studied; the linear association through time (Moggia et al. 2017b) allowed the calculation of the rate of weight loss ($g \cdot d^{-1}$) for each replicate.

To verify the results obtained from the accelerated dehydration of the fruit, the initial and final firmness ($g \cdot mm^{-1}$) were measured using a FirmTech 2 instrument (BioWorks, Wamego, KS, USA), setting minimum and maximum compression forces at 0.15 N and 1.96 N, respectively; the loading cell was adjusted to 6 $mm \cdot s^{-1}$.

The data were studied by an analysis of variance using R version 3.0.0 statistical software R (R Development Core Team, Vienna, Austria, 2008). Means were separated using Tukey's test ($P < 0.05$). A regression analysis was performed to determine the relationship between RH and the rate of weight loss of the fruit at each peaking date.

Results

At each harvest, fruit weight loss was significantly affected by the fruit and RH treatments, as well as the interaction between them ($P < 0.0001$) (Fig. 2).

Weight loss was minimal in each fruit treatment at 96% RH; however, at lower RH, it was 37% to 65% higher than the control when the waxy bloom was removed from the berries, and 27% to 63% lower than the control when the stem scar was sealed (Fig. 2). Except for fruit exposed to 30% RH at the early harvest, water loss was similar between

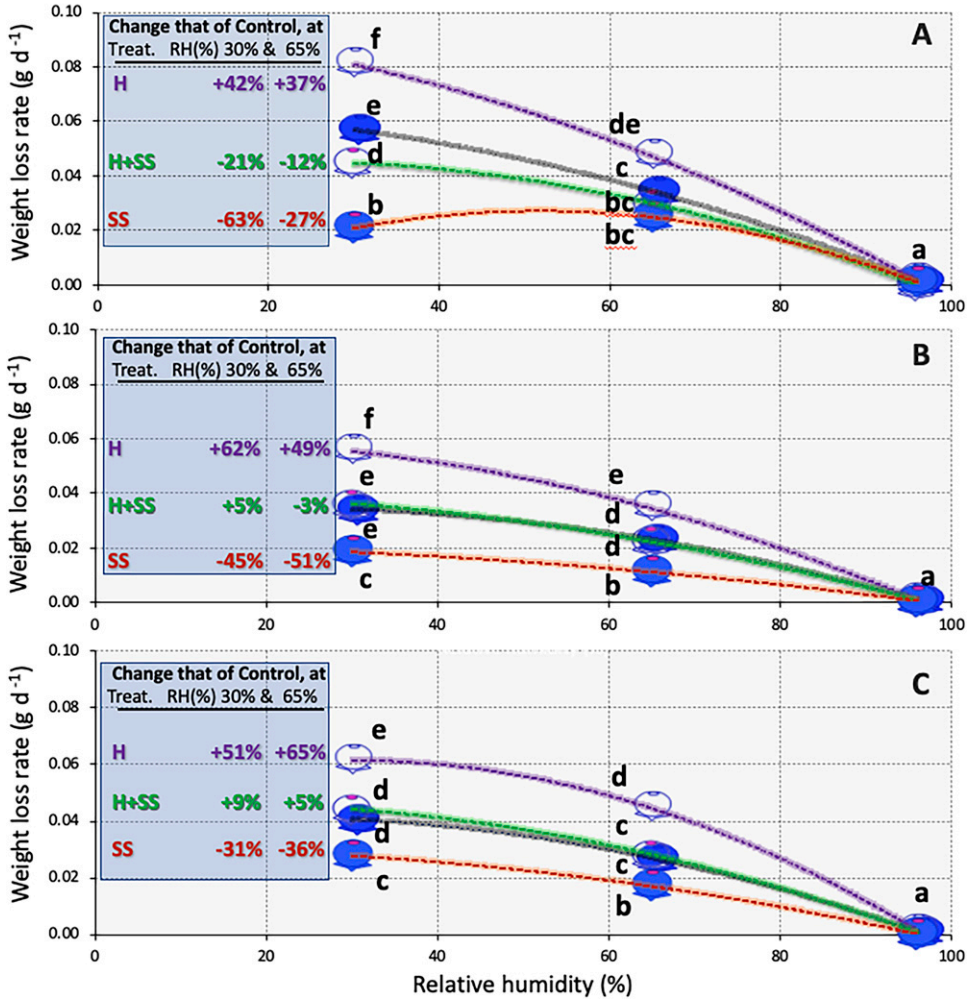

Fig. 2. Weight loss ($g \cdot d^{-1}$) of 'Brigitta' blueberries sampled at early (**A**), peak (**B**), and late (**C**) harvest dates in 2021 to 2022 ($P < 0.00001$, $P < 0.00001$, and $P < 0.00001$, respectively). On each date, the berries were untreated [control (C)], immersed in hexane for 5 s to remove the bloom [hexane (H) treatment], painted with quick-drying nail polish on the pedicel end to seal the stem scar (SS treatment), or immersed in hexane for 5 s and painted with quick-drying nail polish on the pedicel end (H+SS treatment) and then placed in cold storage (1.2 ± 0.7 °C) at three levels of relative humidity for 10 d. Each symbol represents the mean of three replicates. Means were separated at each humidity using Tukey's test ($P \leq 0.05$).

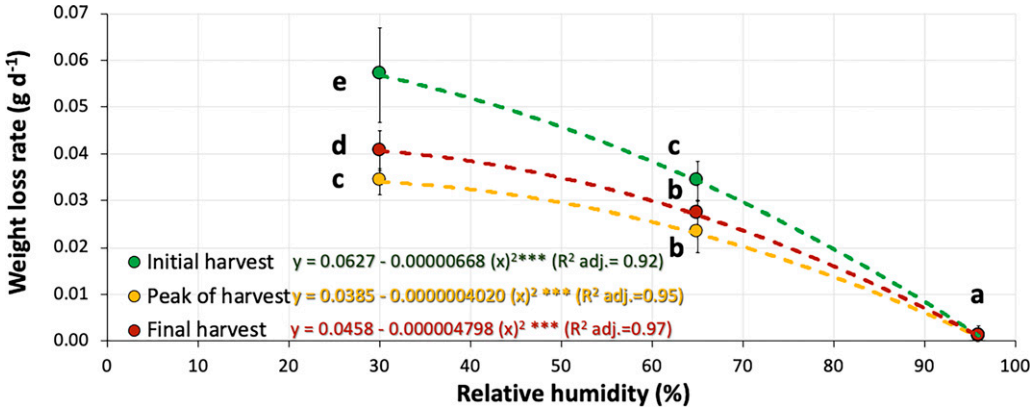

Fig. 3. Weight loss ($g \cdot d^{-1}$) of untreated 'Brigitta' blueberries sampled at early, peak, and late harvest dates in 2021–22. On each date, the berries were placed in cold storage (1.2 ± 0.7 °C) at three levels of relative humidity for 10 d. Each symbol represents the mean of three replicates, and errors bars represent ±1 *SD*. Means were separated at each humidity using Tukey's test ($P \leq 0.05$).

the control and berries treated with a combination of bloom removal and a sealed stem scar.

A regression analysis of the control treatment indicated that water loss at lower RH occurred faster in fruit from the first harvest than in fruit from the peak and final harvests (Fig. 3).

Both firmness at harvest (early: 178.0 $g \cdot mm^{-1}$; peak: 191.2 $g \cdot mm^{-1}$; late: 170.3 $g \cdot mm^{-1}$; data not shown) and at postharvest (Supplementary Fig. 2) showed that the peak harvest had the highest firmness, followed by the first and last harvests. Hexane-treated fruit (hexane treatment and hexane plus stem scar treatment) showed approximately half the firmness of control and stem scar treatment fruit. Stem scar-treated fruit showed differences with the control only for the first and peak harvests, but not for the final picking.

Discussion

Overall, the novel system performed adequately. However, the lower RH level (30%) increased throughout the experiment from 25% to 35%. In this sense, because glycerol was not changed during the experiment, the high flow rate used could have generated a high rate of water evaporation in the glycerol solution, thus affecting the expected glycerol concentration and, consequently, the RH. Therefore, the solutions should be changed/adjusted at least once during the experiment.

As demonstrated by this work, fruit water loss in blueberries was minimal during cold storage when RH was maintained close to 96%. Because maintaining high RH in the entire commercial chain is difficult, fruit water loss in the range of 5% to 7% is considered acceptable during commercial 3-week maritime transport of blueberries (Paniagua et al. 2014; Sargent et al. 2006).

However, as observed during this study, lower RH results in daily water loss, which forces producers to overweigh the commercial units to compensate for dehydration of the fruit during storage and transport. Because each packaging unit (i.e., clamshell) often contains more than 70 berries, random distribution of the berries within the container may limit the holistic understanding of the impact of postharvest treatments or fruit characteristics of a particular harvest on fruit softening. Under normal circumstances, dehydration at the individual level is diluted by the position of each berry relative to the surrounding ones. Therefore, berries at the center of the clamshell interact with a wetter environment than those located more externally. Together with a harvest index that makes it difficult to recognize ripe fruit from overripe fruit, the number of berries in a clamshell accounts for, in part, the difficulty of predicting postharvest softening from measurements of firmness at harvest. The use of DADs can help overcome this difficulty.

Moggia et al. (2017b) reported that weight loss for individual berries is linear over 15 d of cold storage. Therefore, calculations of fruit weight loss can be used as a reliable indicator of fruit dehydration during storage. In the present study, daily measurements of DAD weight proved to be sensitive enough to identify the expected differences among postharvest treatments and harvest dates (i.e., dissimilarities in fruit subjected to different stimuli). For example, when the waxy layer (bloom) of the fruit was removed, water loss increased by up to 65% relative to the control at 60% RH, and by up to 63% at 30% RH. Chu et al. (2017) observed similar results during a study of the waxy layer of blueberries. In contrast, when the stem scar was sealed, water loss declined by 27% to 51% at 60% RH, and by 31% to 63% at 30% RH. This result confirms that the scar is an essential barrier for fruit water loss.

Although changes in fruit water loss were consistent between treatments, picking time was relevant to determining the magnitude of the differences with regard to the control treatment. In this case, the impact of bloom removal on fruit weight loss appeared to be greater at later harvest dates than at the early dates (increased by 37% to 42%, 49% to 62%, and 51% to 65% relative to the control at the early, peak, and late harvests, respectively). Dehydration of the untreated berries, however, was lowest at the peak of harvest, as previously reported by Lobos et al. (2018).

The behavior of firmness after 10 d of forced air storage (Supplementary Fig. S2) is congruent with the results observed for dehydration (Fig. 2). Therefore, at the time of peak harvest, control fruits showed the lowest water loss rates; this situation has been reported previously (Moggia et al. 2022; Moggia and Lobos 2023).

In conclusion, the results of the present work revealed that DADs can be used to characterize preharvest and postharvest stimuli at an individual level and within a short time (10 d); therefore, they may enhance the prediction of fruit behavior during long-term storage of blueberries.

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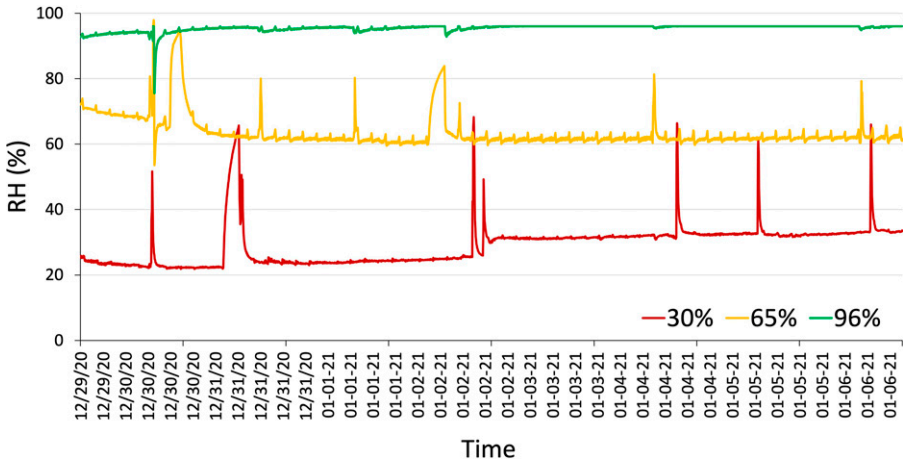
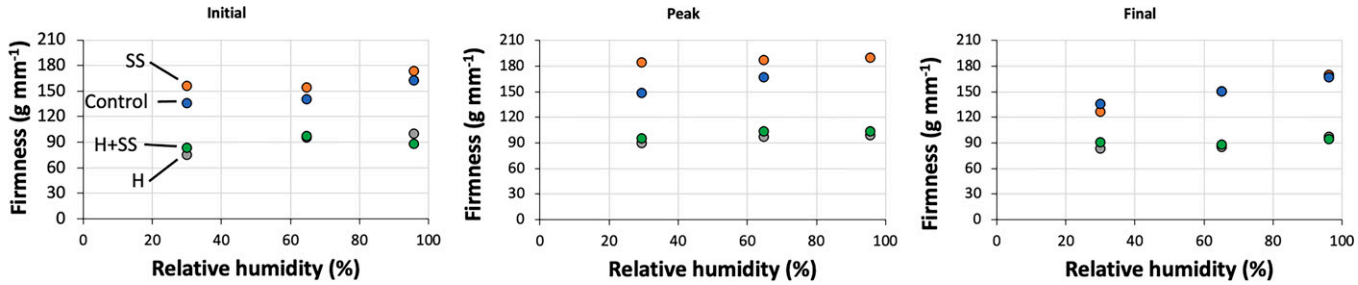
Supplementary Material S1 and S2. 3D image files of the components of Dangler for Accelerated Dehydration (DAD): basket (S1) and body (S2).



Supplementary Fig. S1. Relative humidity recorded during the execution of the experiment.



Supplementary Fig. S2. Firmness after 10 d of forced air storage. On each date, the berries were untreated (Control), immersed in hexane for 5 s to remove the bloom (H), painted with quick-drying nail polish on the pedicel end to seal the stem scar (SS), or immersed in hexane for 5 s and painted with quick-drying nail polish on the pedicel end (H+SS) and then placed in cold storage (1.2 ± 0.7 °C) at three levels of relative humidity for 10 d.