Using Density Measurements to Study the Effect of Excision, Storage, Abscisic Acid, and Ethylene on Pithiness in Celery Petioles

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Abstract. The density of excised 2-cm celery (Apium graveolens L.) petiole segments was highly correlated with a subjective evaluation of pithiness. Loss of density and the appearance of pithiness was stimulated by lengthening the duration of storage, raising the storage temperatures above 0°C, and excising petiole segments. Segments excised from the upper two-thirds of the petiole lost less density during storage than segments excised from the bottom third of the petiole. Segments with initial high densities lost slightly less density during storage at 5°C for 5 weeks than segments that were initially less dense. The extent of pithiness development varied significantly among six cultivars held at 5°C for 2 weeks. Treating whole petioles with 1 µM abscisic acid for 4 days significantly increased density loss. Exposing petiole segments to up to 100 µl·liter⁻¹ ethylene in humidified air for up to 2 weeks at 5°C did not significantly change density over air controls. The loss of density and the development of pithiness in lightly processed celery petioles could be reduced by selecting resistant cultivars, monitoring water stress during growth, using only segments excised from the upper two-thirds of the petiole, and selecting segments with initial high densities.

Materials and Methods

Plant material and storage conditions. Horticulturally mature celery stalks were obtained from local wholesalers and stored at 2.5°C until used. Individual petioles were removed from the stalk...
and either used as entire petioles or as excised 3- or 10-cm-long segments. Only stalks and petioles free of external defects were used. After excision, the stalks or segments were placed in 16-liter glass jars that were ventilated with a flow of humidified, ethylene-free air at 0, 5, 10, 15, or 20°C. After 1 and 2 weeks, stalks were taken out of the jars, the petioles removed from the stalks, and 2-cm sections excised from the center of the top, middle, and bottom regions for density determination. In one study, some petioles were cut into 10-cm segments and stored for an additional week, after which density measurements were made of the middle 2 cm of randomly selected segments. In another study, three 3-cm segments were held in 25 × 100-mm-diameter plastic petri dishes for 2 weeks. All experiments were repeated with similar results.

**Subjective determination of pithiness.** A freshly made transverse cut was used to examine the petiole for visual signs of pithiness. Initially, many petioles were cut and arranged in order of increasing pithiness based on a subjective visual evaluation of each segment. The following scale was derived from these initial observations of the development of whitish areas in the petiole and a score of 0 to 9 was given to petioles based on the extent of the whitish discoloration. A score of 0 = no discoloration; 3 = a few white areas in several locations; 6 = white areas near most of the vascular strands and/or small air spaces; and 9 = large white areas and/or many air spaces.

**Density determination.** Celery petiole segments were trimmed to the central 2 cm before being weighed to the nearest milligram. Segments were then pierced with a thin 3-cm-long stainless-steel pin. A stand was constructed that held the end of the pin and could be used to lower and completely immerse the segment in a beaker of water on a balance. The increase in weight upon immersing the petiole segment in the water equals the weight of the displaced water. The volume of the celery segments was calculated from this weight measurement by dividing the weight of the displaced water by the density of water. The temperature of the water was measured and the density of water obtained from a table of critical values. The density of the petiole segment was calculated by dividing the weight of the petiole by the calculated volume.

**ABA applications.** Petioles were cut from stalks and placed in 600-ml beakers containing 100 ml of 0, 1, 10, and 100 µM ABA. The beaker and tissue were placed at 20°C under constant dim fluorescent light. The solutions were replaced as needed. After 96 h, 2-cm segments were excised from the middle of the top, middle, and bottom third of the petioles. The density of the petiole segments was determined as described above.

**Ethylene exposure.** Excised 3-cm celery petiole segments were placed in 25 × 100-mm-diameter plastic petri dishes in 16-liter glass jars containing concentrations of 0.0, 0.1, 1.0, 10, 100, and 200 µl·liter⁻¹ ethylene in air. An ethylene scrubber was included in the ethylene-free jar. A 100-ml beaker containing KOH pellets was included to absorb CO₂. The jars were sealed and placed at 5°C. Periodic measurement and introduction of air insured that the O₂ concentration remained above 15%. After 2 weeks, segments were removed and their densities determined as described above.

**Results and Discussion**

**Density and subjective pithiness score.** Many 2-cm-long petiole segments were excised from many stalks of celery and ranked on the basis of visual signs of pithiness. Three to six segments were selected for each of the 9 pithiness ratings (0 to 8) for density measurements. Objective density measurements were highly correlated with subjective visual determinations of pithiness (Fig. 1).
The relationship between the subjective pithiness scores of 0 to 8 and the density of the segments is given by the following linear equation: density = 0.9993 – [0.0036 x (subjective score)] with an $r^2$ of 0.89. The density of segments with a subjective score of 9 was highly variable and appeared to encompass a wide range of levels of aerenchyma development. The density values for segments with a score of 9 deviated from the linear relationship between density and subjective evaluation and were therefore not included in the regression analysis.

The breakdown in the relationship between density and our subjective visual evaluation of pithiness at the highest subjective score of 9 was possibly because the presence of air spaces in these segments, but not the size of the air spaces, was a distinguishing characteristic of this rating. A small change in the number or size of internal air pockets would significantly alter the calculated density of the segment without significantly altering the subjective score. Also, some of the air spaces in petiole segments scoring 9 were sufficiently large that water entered the tissue when it was immersed for the volume measurement.

**Changes in density with time and temperature.** The density of excised petioles decreased with increasing temperature from 0 to 20°C (Fig. 2). Over the 18 days of the experiment, the decrease was slight in segments held at 0°C. The density of petiole segments was similar for tissue held at 0 and 5°C for 9 days. After 9 days, the loss of density in tissue held at 5°C significantly increased over that of tissue held at 0°C, so that by day 18 the density of the 5°C tissue was significantly less than segments held at 0°C. In contrast to this delay in density loss at 5°C, the loss of density was immediate and rapid for segments held at 10 to 20°C. However, after 7 days at 20°C or 9 days at 15°C, the density of these segments actually started to increase significantly. A visual inspection of these segments showed that many were senescent (i.e., yellowing) and partially translucent (i.e., water-logged). The intrusion of water into the aerenchyma tissue would account for the increase in density as air spaces were filled with water.

The time it took the segments to decrease to a quality rating of 3 (e.g., to a density of 0.99 g·ml$^{-1}$) represented the useful storage life and decreased in a log-linear fashion, with the length of storage being halved by each 5°C rise in temperature. This decline in quality versus temperature corresponds to an average Q$_{10}$ of 3.85. The length of storage is given by the following equation: log of the storage life in days = 1.43 – 0.0586 × temperature, with an $r^2$ of 0.89 over the range of 0 to 20°C.

The rate of density loss for 3-cm segments held at 0, 10, and 15°C, after storage at 0°C for 5 days was similar to that of segments held at these temperatures immediately after excision (Fig. 3). After 7 days at the transfer temperatures (i.e., on day 12), the densities were statistically indistinguishable from the 7 day values shown in Fig. 2 for segments held at 0, 10, and 15°C.

While the overall pattern of density loss was unaffected by holding the segments at 0°C for 5 days before transferring them to 10 or 15°C (Fig. 3), the storage life of these segments was about 1 day less than that of segments held at 10 or 15°C immediately after excision (Fig. 2). Obviously, some small amount of quality loss had occurred during the 5 days holding at 0°C.

**Effect of excision on density loss.** Excision of petiole segments may have encouraged density loss in comparison to entire petioles. Segments excised from entire petioles and immediately measured had densities of 1.00±0.003 g·ml$^{-1}$. In comparison, segments that were held for 7 days at 10°C as 3-cm segments had an average density of 0.976±0.005 g·ml$^{-1}$, while segments excised from intact petioles that were held for 7 days at 5°C had a significantly higher density of 0.984±0.004 g·ml$^{-1}$. It appears that density was better maintained in intact petioles than in segments.

We also noted that the segments weighed slightly less after storage than immediately after excision. However, a loss of weight (i.e., a loss of water) would not have significantly affected the density of the segment unless the volume of the segment changed less than the volume of the water lost. This possibility was tested by rapidly dehydrating some segments to give an average weight loss of about 5%. The initial weight and density of the segments were 3.007 ± 0.010 g and 0.998 ± 0.003 g·ml$^{-1}$, respectively. After a weight loss to 2.850 ± 0.017 g in 4 h at room temperature, the density had changed to 0.996 ± 0.008 g·ml$^{-1}$. Although the weight loss was more rapid than occurred during the week of storage, it suggests that weight loss per se was not the cause of the reduction in the density of the stored segments versus the intact petioles.

**Effect of storage on density loss.** The density of many freshly excised segments was measured and 60 segments were selected that had densities roughly distributed between 1.00 to 0.95 g·ml$^{-1}$. The 60 segments were ranked by their initial density and a linear regression equation fit to the data (Fig. 4A). The densities were again measured after 2 weeks of storage at 5°C.

The initial density of excised petiole segments was significantly correlated ($r^2 = 0.79$) with their density after 2 weeks of storage (Fig. 4B). However, the extent of the changes in density was only slightly related to the initial density of the segment. This propensity for segments with lower initial densities to have greater

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Fig. 4. The density of 2-cm petiole segments after excision and after 2 weeks of storage at 5°C. (A) Density of the segments immediately after excision (■) and after 2 weeks ( ● ) of storage. The data are arranged in descending order of their initial densities. (B) Relationship between the initial and final densities. The linear regression equations are given for each set of data.
density loss during storage was shown by the significantly different slopes of the linear regression equations. The slope of the equation fitted to the densities of the stored segments (i.e., $-0.00106 \text{ g/ml/ranking}$) was larger than the slope of the equation fitted to the initial densities (i.e., $-0.00089$). The differences in the slopes means that, as the rank number of the segment increased (i.e., as their initial density decreased), the difference between the two equations (i.e., the change in density during storage) increased. This implies that petiole segments low in initial density lost more density during storage than segments with higher initial densities. While there was a great deal of variability in the data, this relationship was confirmed by the data. For example, the average change in density for the 15 segments that had the highest initial densities ($0.9928 \pm 0.0025 \text{ g/ml}$) was $0.0226 \pm 0.0092 \text{ g/ml}$, while the average change in density for the 15 segments with the lowest initial densities ($0.9569 \pm 0.0072 \text{ g/ml}$) was $0.0294 \pm 0.0103 \text{ g/ml}$.

The factors governing density loss in excised celery petiole segments appear to vary considerably among the segments and the predictive value of a single density measurement is subject to a great deal of variability. While the location from which the segment was excised was not retained during these experiments, other studies (Figs. 7 and 8) indicated that segments excised from the upper two-thirds of the stalk were less prone to rapid density loss than were segments excised from the basal portion of the petiole.

**Differences in density among cultivars.** There were significant differences among the six celery cultivars in the initial density of excised segments and the density of these segments after storage as 10-cm-long segments at 5°C for 2 weeks (Fig. 5). Segments from ‘Matador’, ‘Napoleon’, and ‘52-70R’ had initial densities at about 1.002 g/ml, while segments from ‘A865’, ‘Summit’, and ‘50-70HK’ had somewhat lower densities at about 0.999 g/ml. ‘50-70HK’ lost the least density ($0.010 \text{ g/ml}$) followed by ‘Summit’ ($0.014 \text{ g/ml}$). ‘52-70R’, ‘Matador’, and ‘Napoleon’ lost similar amounts of density ($0.026 \text{ g/ml}$). The greatest amount of density lost was from ‘A865’ ($0.031 \text{ g/ml}$).

Although the cultivars were grown in a similar location and under similar conditions, their differences should be viewed with caution since the preharvest factors of drought stress and mechanical injury, which are known to significantly influence the development of pithiness, were not actively monitored nor controlled in these experiments. These data reveal relative differences in susceptibility to develop pithiness among cultivars rather than the absolute level of pithiness that will develop. Under different climatic or handling conditions, the cultivars may respond differently.

**Effect of ethylene.** Exposure to ethylene concentrations that could conceivably be encountered during processing and marketing, e.g., up to 20 μl·liter$^{-1}$, had no significant effects on density loss during storage for 2 week at 5°C (Fig. 6). However, there appeared to be a progressive decline in density as ethylene concentrations increased from 20 to 200 μl·liter$^{-1}$, with a significant reduction in density occurring at 200 μl·liter$^{-1}$. However, tissue exposed to 200 μl·liter$^{-1}$ ethylene in air were rapidly senescing, and it is difficult to conclude if the ethylene effect was induced through senescence or aerenchyma formation.

When the position in the petiole from which the segments were excised was factored into the analysis, it became evident that 100 μl·liter$^{-1}$ ethylene in air caused a consistent reduction in density (Fig. 7). These data also show that segments excised from the base of the petiole were generally less dense than segments excised from the top of the petiole. This suggests that the loss of density and

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**Fig. 5.** Density of 2-cm petiole segments excised from whole petioles of six celery cultivars immediately after harvest and after 2 weeks of storage as 10-cm long segments at 5°C. The vertical line above each bar represents the standard deviation.

**Fig. 6.** Effect of 2 weeks of storage at 5°C in various concentrations of ethylene on the density of 3-cm petiole segments. The vertical line above each bar represents the standard deviation.

**Fig. 7.** Effect of 2 weeks of storage at 5°C in air or 100 μl·liter$^{-1}$ ethylene on the density of 3-cm petiole segments excised from the top, middle, and bottom third of whole stalks. The vertical line above each bar represents the standard deviation.

**Fig. 8.** Effect of 2 weeks of storage at 5°C in air or 100 μl·liter$^{-1}$ ethylene on the density of 3-cm petiole segments excised from the top, middle, and bottom third of whole stalks. The vertical line above each bar represents the standard deviation.
the development of pithiness developed earliest in the basal portion, as would be expected from an ontological perspective.

**Effect of ABA.** No significant differences were observed among segments excised from the top portions of petioles treated with the lower concentrations of ABA (Fig. 8). Segments excised from the middle and bottom portion of petioles treated with 1 and 10 µM ABA were less dense than the controls. The 100 µM ABA concentration caused such profound senescence that tissue collapse allowed water-logging, which resulted in an actual significant increase in the density of segments excised from all portions of the petiole. The ability of ethylene (Fig. 7) and ABA (Fig. 8) to induce density loss was greater in the bottom third of the petiole than in the upper two-thirds.

**Predicting the development of pithiness.** Methods of predicting the onset of pithiness could be commercially used to evaluate celery before or after processing. Material likely to become pithy could be diverted to other uses. Initial density measurements were significantly correlated with subsequent changes in density and shelf life. Separating lightly processed celery stalks into specific density classes could provide a means to control quality during marketing. However, measuring the density of individual petiole segments is time consuming and impractical for large-scale screenings. Separating segments based on their ability to float or sink in aqueous solutions of certain densities could provide a rapid means to screen large amounts of tissue.

The following procedures could be used to reduce the propensity of harvested and/or lightly processed celery petioles to develop unacceptable levels of pithiness during marketing: 1) select a cultivar that exhibits resistance to the development of pithiness; 2) monitor growing conditions to ascertain the level of water stress the growing plants are exposed to; 3) use only segments excised from the upper two-thirds of the petiole; and 4) select segments with a high initial density.

**Literature Cited**


