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A Rapid Method for Evaluating Curing Progress in Sweet Potatoes[1]

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Abstract. **Freshly harvested sweet potatoes [*Ipomoea batatas* (L.) Lam. c. Jewel] are cured by holding them at 80–95% relative humidity and 29.4°C for 5 to 7 days. Curing heals wounds inflicted during harvest, thus minimizing loss from microbial decay during subsequent storage. The wound healing process was followed by applying a saturated solution of phloroglucinol in strong acid (PG) to the underside of detached wound tissue and scoring the intensity of the color developed. Microscopic examination of companion tissue with PG showed that color intensity was due to the layers of cells in which PG positive material was deposited. Wound periderm formation was observed to occur simultaneously with development of the most intense color, indicating that the test may be useful in the evaluation of curing progress.**

More than half of the sweet potato crop produced in the United States is sold on the fresh market. If this vegetable is to be available for most of the year, then the roots that are harvested from August through mid-November must be stored for 6 to 8 months. The ability to store roots for long periods of time with minimal loss from decay is of such economic importance to the industry that considerable research efforts have been directed toward achieving that goal (2). Current recommendations include harvesting roots before a killing frost, immediately placing them in curing conditions (29.4°C, 85–90% relative humidity) for 4 to 7 days, and storing them between 12.7 and 15.5° with a relative humidity of 85 to 90% (10). The purpose of curing is to rapidly heal any damage (cuts, bruises, etc.) inflicted during harvest and thus to minimize decay and water loss.

The effect of various temperature and humidity regimes on the rate of wound healing in sweet potatoes has been the subject of many studies (1, 5, 6, 8, 9). Although there is some disagreement as to what conditions preclude wound healing, there is agreement on the chronology of wound healing under favorable conditions.

After immediate placement of a wounded root into curing conditions, the first change observable on a cellular level is the desiccation of several layers of parenchyma cells at the wound surface. Soon thereafter, the walls of cells directly beneath the desiccated cells begin to thicken. Artschwager and Starrett (1) differentiated these cells by their capacity to absorb ammoniacal crystal violet stain in contrast to normal parenchyma cells, which remain unaffected. This newly deposited material on the cell wall was designated as suberin. Later, while evaluating the progress of suberization in the parenchyma cells beneath the wound surface, McClure (5) reported that the suberized cells had a much stronger affinity for a saturated solution of phloroglucinol (PG) in 18% HCl than for the stain used by Artschwager and Starrett (1). This led McClure (5) to question whether suberization or lignification was occurring. Suberization (or lignification) progresses to deeper-lying cells and ceases when a wound cork layer of cells begins to form. The wound cork region increases until several layers of wound cork are produced. At this time, the rate of wound cork formation decreases dramatically, indicating that healing is complete.

Although it is not known how many layers of wound cork cells are necessary to prevent infection, it is known that the combination of suberized cell walls and wound cells is an effective mechanical barrier to microbial infection (4, 5, 9) as is the uninjured periderm. If a simple method were available to determine when wound healing was complete, it could serve as an objective indicator for completion of the curing process. Heretofore, such a determination required specialized skills and equipment as well as considerable sample preparation time, and thus was not feasible. Proper curing requires expensive facilities and sizeable energy input. Lack of a rapid, objective method to determine when roots may be safely removed from curing has led to the practice of recommending a spread of several days with the individual deciding subjectively (sprouting, appearance, etc.) when the roots are cured. It is the purpose of this paper to propose a rapid objective method using a color test to evaluate curing progress.

Materials and Methods

Color test. 'Jewel' roots were harvested in early September 1981. Beginning on the harvest day and continuing daily over a 7-day period, 5 roots per day were wounded with a single edge razor blade by removing a circular patch of tissue about 1 cm in diameter and 1 to 2 mm in depth from the mid-region of each root and then placed in an environmental chamber (Forma model no. 39412-1) maintained at 29.5°C and 85% relative humidity (RH). At the end of 7 days, all roots were removed. The wounds were excised from each root by slipping a razor blade under the wound and separating the wound from the healthy tissue. Wounds from roots subjected to the environmental chamber conditions for 1 to 3 days were difficult to remove.

To perform the color test, 5 drops of a saturated solution of phloroglucinol (PG) in 18% HCl were placed on the detached wound surface which was previously adjacent to the healthy tissue. After 10 minutes, the tissue was blotted and its color was scored visually to the nearest whole number using a 1 to 4 scale.

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The scale was as follows 1 = no color to faint pink around the edges; 2 = pink; 3 = red; 4 = deep reddish-purple. Using the wounds from this preliminary study, a color print was made which illustrated the scale (Fig. 1). This photograph was used to score the extent of color development in the study described below.

Treatments. 'Jewel' sweet potato roots were harvested on Sept. 21, 1981, and Oct. 21, 1981, from the Central Crops Research Station in Clayton, N.C. Only healthy, uninjured roots of marketable size (U.S. no. 1 and 2) were selected for the study. The side of each root was wounded with a single-edge razor blade as described above. The roots harvested and wounded on Sept. 21, 1981, were divided into 4 separate groups of 50 roots each and placed in rectangular (47 × 30 × 34 cm) storage boxes. Each box was then placed in one of the following environments on the day of the harvest: (A) 29.5°C, 83 to 87% RH (Forma Scientific environmental chamber, model no. 39412-1); (B) 21.5 to 24.5°, 40 to 60% RH (ambient laboratory conditions); (C) 27.5 to 29.0°, 78 to 80% RH (Central Crops Research Station curing room); and (D) 17.0 to 18.2°, 68 to 71% RH (Central Crops Research Station storage room). The roots harvested and wounded on Oct. 21, 1981, were divided into 2 groups of 50 roots each. Each group in storage boxes was placed into one of the following environmental treatments on the day of harvest: (E) identical to (A) and (F) 21.0 to 24.0°, 40 to 58% RH (ambient laboratory conditions). All temperature and humidity measurements were recorded daily with a psychrometer (Psychro-Dial, Environmental Tectronics Corporation, model CP-147).

The response of wound tissue samples to the different environmental treatments was evaluated for a total of 14 days subsequent to each harvest. During the sampling periods, the optimal curing groups A, C, and E were subjected to their respective environmental treatments for 7 days. The remaining, unsampled roots in these groups were held at 17.0°C, 65% RH for an additional 7 days until all sampling was completed. Five roots per day (1 wound per root) were sampled for each of groups A, C, and E at 3, 4, 5, 7, 10, and 14 days after wounding. Five days after wounding, 10 roots from each of groups A, C, and E were removed from their respective environmental treatments and held at 17°, 65% RH, until sampled after 2 and 5 additional days (5 roots each). The suboptimal groups B, D, and F were subjected to their respective environmental treatments for 14 days. Five roots per day (1 wound per root) were sampled for each of groups B, D, and F at 7, 10, and 14 days after wounding.

Sampling. Wound tissue (about 1 cm^3) was sampled by dividing the wound from each root into 2 equal portions. One half of the wound from each root was excised by slipping a razor blade under the wound tissue and separating the wound from the healthy tissue. The undersurface of this half of the wound was treated immediately with 5 drops of phloroglucinol solution and scored for color development after 10 minutes. The remaining half of each wound was excised by removing a 5-mm cube of tissue which included both wound tissue and healthy tissue. These 5-mm cubes were fixed in 3% glutaraldehyde in 0.15 M sodium phosphate buffer, pH 6.0, for anatomical observation with the light microscope.

Microscopy. Fixed blocks of wound tissues were dehydrated with an ethanol-teritiary butyl alcohol series, embedded in paraffin (3) and sectioned at 15μm on a rotary microtome. The sections were examined with the light microscope after staining with the saturated phloroglucinol solution. For each wound, the number of layers of red-staining suberized cells (phloroglucinol positive) and wound cork cells were counted and tabulated.

Statistical analysis. Data obtained from optimal treatments A, C, and E were pooled and subjected to an analysis of variance procedure (ANOVA) and the Waller-Duncan K-ratio *t* test (7). These procedures provided least significant differences (LSD) by which appropriate means were compared. Pooled data from suboptimal treatments B, D, and F were analyzed in the same manner.

Results and Discussion

Color test. Earlier work in this laboratory demonstrated that the degree of suberization could be determined by the color resulting from the application of the phloroglucinol-HCl reagent (PG) to the underside of a detached wound. This is possible because after several days in curing conditions, the layers of suberized cells are easily detached from those parenchyma cells which are beginning to differentiate into wound cork cells. The 4-point scale was established and used for the storage study described below. It should be noted that in this test 5 roots were used for each day and in every case wounds from all 5 roots gave the same color with PG, indicating that under favorable conditions suberization is both uniform and rapid. We also observed that after the reddish-purple color has been attained, no further color change occurs.

Curing studies. Sweet potatoes are cured when wounds are healed. There are no criteria for determining when a wound is healed except that both suberized and wound cork cells be present. This portion of the study was designed to relate color score as we have defined it to suberization and wound cork formation. Since current recommendations call for an optimum curing period from 5 to 7 days at 85°F and 85 to 90% RH (10), 7 days was the maximum time our roots were cured (groups A, C, and E).

We found (Table 1) that the color score reached "4" at 5 days and did not change thereafter for optimally cured roots (groups A, C, and E). The rate of color development was similar

Table 1. Color score[z], number of layers of suberized cells and number of layers of wound cork cells in 'Jewel' sweet potatoes cured under optimal conditions.[y]

Days cured	Color score[v]	Layers of suberized cells[u]	Layers of wound cork cells[u]
3	2	1.4C	0E
4	3	2.6B	0E
5	4	4.2A	0.6D
5 + 2[x]	4	4.1A	2.6C
5 + 5[x]	4	4.3A	3.2B
7	4	4.3A	3.3B
7 + 3[w]	4	4.3A	4.2A
7 + 7[w]	4	4.4A	4.2A
$LSD_{.05}$	---	0.3	0.3

[z]Color developed when wound tissue treated with phloroglucinol reagent, 2 = pink; 3 = red; 4 = reddish-purple.
[y]Pooled data from treatments A, C, and E with 50 roots per study. Results tabulated from mean values for 15 roots per day (78–89% RH, 27.5 to 29.0°C).
[x]Five days curing + days stored at 17.0°C, 65% RH.
[w]Seven days' curing + days stored at 17.0°C, 65% RH.
[v]Statistical analysis not possible due to no variability of daily replicates.
[u]Numbers in vertical columns are different ($P \leq 5\%$) where letters are different

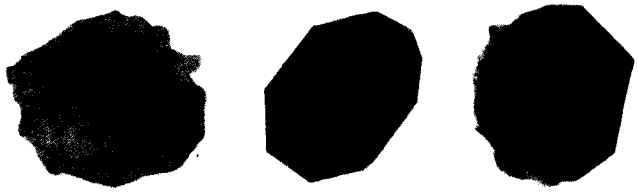

Fig. 1. Phloroglucinol-HCl color test (from left to right): 2 = pink, 3 = red, 4 = reddish purple. The color score of each of these 3 pieces of excised wound tissue from 'Jewel' sweet potato roots is directly related to the number of layers of suberized cells present. 2×.

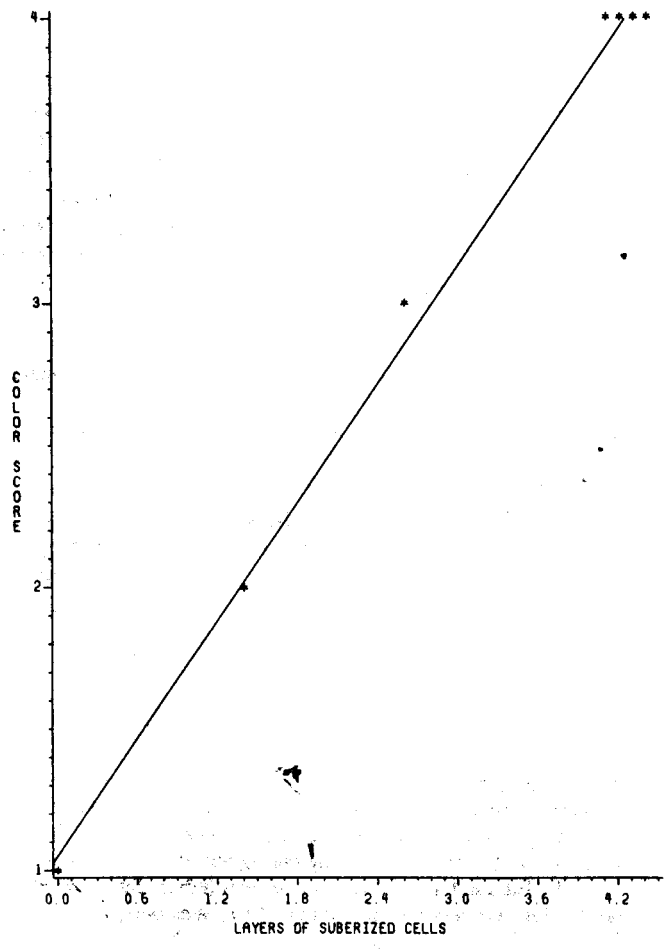

Fig. 2. Linear regression of color score on layers of suberized cells. Each point represents 15 roots.

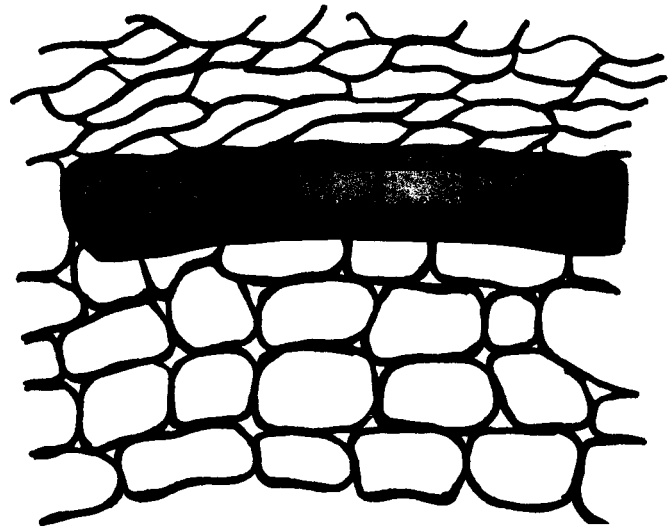

Fig. 3. Schematic diagram of a sweet potato wound cross-section stained with phloroglucinol-HCl. The 1 to 2 layers of red-staining suberized cells correspond to a score of 2 in the color test.

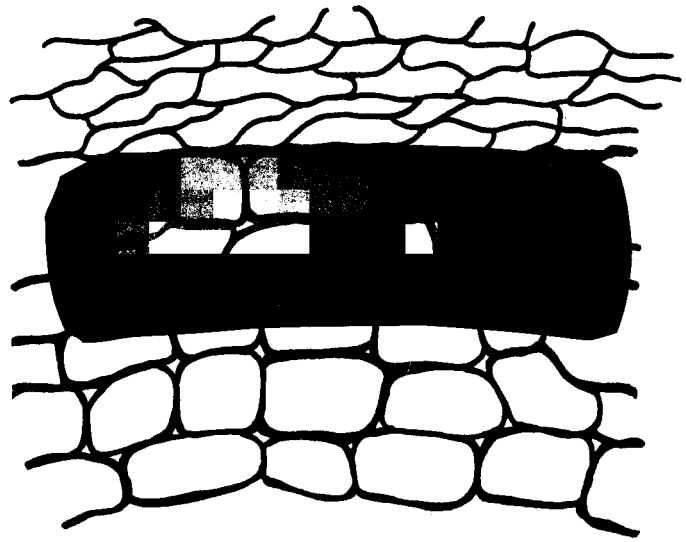

Fig. 4. Schematic diagram of a sweet potato wound cross-section stained with phloroglucinol-HCl. The 2 to 3 layers of red-staining suberized cells correspond to a score of 3 in the color test.

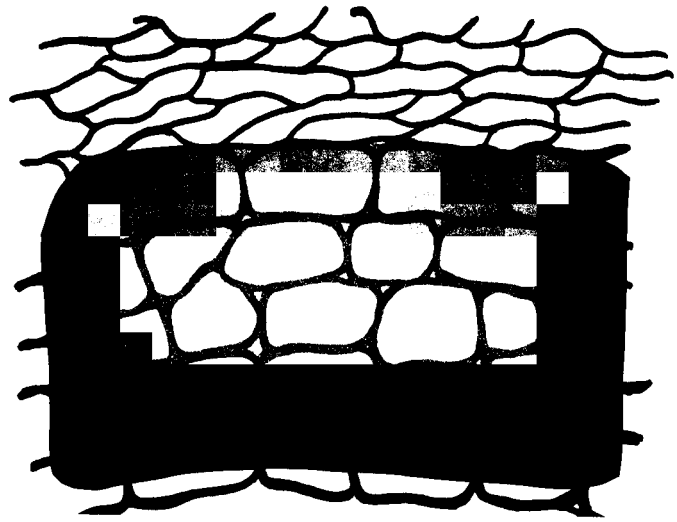

Fig. 5. Schematic diagram of a sweet potato wound cross section stained with phloroglucinol-HCl. The 4 to 5 layers of red-staining suberized cells correspond to a score of 4 in the color test.

to that which was observed when the color scale was developed. There was no variability observed for the replicates which were evaluated on any given day, and thus, it was not possible to apply statistical methods to the color scores. When we examined the companion tissue sections microscopically, we did find some variability in the number of layers of both suberized cells and wound cork cells. Layers of suberized cells increased until there were about 4 to 5 layers present, which corresponded to the highest color score of "4" (reddish purple). If the number of layers of suberized cells vs. the color score are plotted (Fig. 2), it is evident that the 2 are highly correlated ($r = 0.99$; $P \leq 5\%$). Photomicrographs (Fig. 3, 4, and 5) illustrate this relationship between the number of layers of suberized cells and the color scores. The "2" is pink and has 1 to 2 layers of suberized cells. The "3" is red and has 2 to 3 layers, while the "4" is reddish-purple and has 4 to 5 layers.

When roots were removed from the curing chamber after 5 days and placed under storage conditions (group A), the wound cork layers continued to increase, going from 0.6 layers to 2.6 layers after 2 days in storage and increasing to 3.2 layers after 5 days' curing plus 5 days' storage. Roots cured the full 7 days developed an additional 0.9 layer of wound cork cells after 3 days' storage and no further increase after 7 days' storage (Table 1). These results are similar to those of Strider and McCombs (8), who noted in 9 cultivars that wound cork production began several days after curing started, increased rapidly for about 4 days, and then very gradually increased during storage. These workers did not evaluate the suberization which preceded wound cork production. Our data show that if wounds are cured long enough to initiate wound cork production (i.e., suberization complete), then additonal wound cork layers will be produced to complete wound healing even if the roots are removed to a cooler environment for storage.

A second goal of the storage study was to evaluate the rate of wound healing under suboptimal curing conditions. The pooled data (Table 2) from 3 groups (B, D, F) indicated that these rates were slower. We were able to analyze statistically the color data in this study because replicates for a given day had not developed a uniform color. At 7 days, both the color score and the layers of suberized cells were approaching the maximum values obtained in the roots (Table 1). However, the wound cork had just begun to form. After 3 more days, the wound cork had increased by almost 3 layers (Table 2). The layers then increased by only 0.3 of a cell layer in the final 4 days of the study. The roots held in the suboptimal storage conditions had the capacity to heal small, razor-cut, lateral wounds as well as optimally cured roots, but at a slower rate. In both cases, visible changes slowed significantly when 4 to 5 layers of both suberized and wound cork cells had been formed, which indicated completion of wound healing.

Table 2. Color score[z], number of layers of suberized cells and number of layers of wound cork cells in 'Jewel' sweet potatoes cured under suboptimal conditions.[y]

Days cured	Color score[x]	Layers of suberized cells[x]	Layers of wound cork cells[x]
7	3.6B	3.7A	0.5C
10	3.8A,B	3.8A	3.4B
14	4.0A	4.0A	3.7A
$LSD_{.05}$	0.2	N. S.	0.2

[z]Color developed when wound tissue treated with phloroglucinol reagent, 3 = red; 4 = reddish purple.
[y]Pooled data from treatments B, D, and F with 50 roots per study (40–71% RH, 17 to 24.5°C). Results tabulated from mean values for 15 roots per day.
[x]Numbers in vertical columns are different ($P \leq 5\%$) where letters are different.

Conclusions

Curing of sweet potatoes by holding them under conditions of high temperature and humidity is practiced primarily to increase storability. The mechanism by which curing is able to retard microbial decay and weight loss is by promoting the wound healing process. This study has shown that one of the first observable cellular changes is suberization (or lignification) of several layers of cells below the wound surface. The cells directly under the suberized layers then begin to divide, and subsequently, a continuous layer is formed below the suberized cells. This continuous layer functions as a cork cambium and continues to divide, giving rise to a layer of wound cork cells. For small, razor-cut, lateral wounds, the observable changes greatly decrease when 4 to 5 layers of both suberized cells and wound cork cells have been formed. We found that when wound cork initiation is begun and the preceding suberization is complete, production of wound cork continues, even if roots are removed from the curing environment and held under storage conditions. This study also demonstrated that suberization can be evaluated by removing the wound, applying PG, and scoring the color.

For small, razor-cut, lateral wounds, the suberization is complete, and wound cork production is initiated when 4 to 5 layers of suberized cells are formed. This corresponds to the reddish-purple score of "4" on the color test. Therefore, the following method is suggested to evaluate the curing progress of sweet potatoes, based on the wound healing process: On the day curing is to begin, a group of roots are wounded with a single-edge razor blade by removing a circular patch of tissue about 1 cm in diameter and 1 to 2 mm in depth from the mid-region of each root. These wounded roots are placed at several locations in the curing room. Beginning at 3 days, wounds from the test roots are excised, PG is applied, and a color score is assigned. This procedure should be repeated daily until all wounds have reached the reddish purple stage. At this time, the roots may be moved to storage.

Because of the variability in the environments of different curing facilities it is quite likely that the recommended curing period of 5 to 7 days may be too long in some cases and too short in others. The test we propose allows the shipper to evaluate the progress of curing in his curing house based on the progress of wound healing. It is possible that close monitoring of the curing process will eliminate under and over curing and will result in economic gain due to decreased energy costs and increased storage stability.

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