



SUPERFICIAL SCALD RISK ASSESSMENT ASSAY FOR APPLES

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Superficial Scald Risk Assessment Assay for Apples

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Aim

Rank apple storage rooms for the risk of superficial scald development by quantifying the conjugated trienols (oxidation product that causes scald symptoms) in the peel of a representative sample of fruit to aid in decision making during cold storage of apples. This assay could have benefits for organic fruit – where scald-mitigating treatments are limited – and for conventional fruit where the effectiveness of scald-mitigating treatments can be validated and corrective actions taken.

Introduction

Superficial scald is a major physiological storage disorder in apples which limits storage potential of the fruit. It is characterized by necrosis of the first 4–6 hypodermal cell layers of the fruit (Bain 1956), and caused by the production of reactive oxygen species in chloroplast-containing cells under stress (Lurie and Watkins 2012). Scald development is affected by a number of pre- and postharvest factors, including: light exposure and temperature during growth, fruit maturity at harvest, storage temperature and atmosphere, and ethylene exposure (Rudell and Mattheis 2009).

The disorder is linked to cold stress during the initial part of cold storage, but symptoms may only develop months into cold storage or the supply chain. Symptoms are associated with α -farnesene oxidation in the peel that forms various conjugated trienols (CTs) (Rowan et al. 2001). Apple postharvest mitigating treatments for superficial scald include the antioxidant diphenylamine (DPA), ultra-low oxygen controlled atmosphere storage, and the ethylene inhibitor 1-methylcyclopropene (1-MCP).

Scald Risk Assessment using Biomarkers

This assay is suitable for assessing superficial scald risk from samples up to 3 months in cold storage, which can predict relative scald risk beyond 6 months – provided the storage environment is not changed. To date, this assay has only been tested for use with ‘Granny Smith’ and ‘Delicious,’ although we expect, with careful validation, it will be effective for other superficial scald-susceptible cultivars, too.

It is most effective for ranking rooms for risk of scald development and identifying rooms with unsatisfactory scald mitigation. It is not recommended for determining lot-to-lot risk within the same room at this stage, but this warrants investigation.

For the assay, natural apple wax is extracted using hexane and conjugated trienols (CTs) measured at 281 nm using a UV-Vis spectrophotometer. A similar model, using absorption maxima at 258 nm and 281 nm to estimate trienol level and scald risk, has been proposed by researchers in Spain (Bordonaba et al. 2013).

Materials

Safety Equipment

- Standard safety equipment: nitrile gloves, lab coat, safety goggles
- Respirator or ventilation (e.g., fume hood) appropriate for organic solvents

Sample Preparation

- Peeler
- Marker
- Cutting board
- 4 mm biopsy punch

Analysis

- Hexane (reagent grade)
- 1 L Schott bottle
- 2x Micropipettes (100 μ L and 1,000 μ L)
- 2 mL microcentrifuge tubes
- Microcentrifuge tube rack
- Low volume quartz cuvette (minimum 2)
- Vortex mixer

Supplier information is provided in the Equipment section.

Method

Technicians performing this assay should have some tertiary education and experience with laboratory procedures. It does require handling of an organic solvent, quartz cuvettes, and a spectrophotometer, so dexterity is required. A member of management should interpret the results.

Step 1. Collect Sample Fruit

Best results can be obtained if doorway samples are placed in the room at the same time as the bins from a specific orchard. In this way, the samples receive the same treatment as the fruit in the bins. Samples must be collected from the CA room only by qualified personnel fitted with an approved breathing apparatus and trained to enter CA rooms. It is recommended that each sample contains three (3) fruit, and each lot has at least three (3) samples per sampling event. Lots should be tested on (i) day of harvest, (ii) 2 weeks, (iii) 4 weeks, (iv) 6 weeks, and (v) 12 weeks. You will therefore need a minimum of 45 fruit for each room. Fruit must be representative of the lot and free from defects.

Step 2. Peel Apple Fruit

- Take one (1) peel slice from every fruit of the same treatment avoiding sun damaged or exposed areas. The peel should be taken from stem to calyx (Figure 1).
- Place all of the peels for each sample on the cutting board (Figure 2).

Step 3. Collect Discs of Peel

- Use a 4 mm biopsy punch to sample an equal amount of discs (about 6 discs per peel) from every peel in the sample set (Figures 3 and 4). Replace the biopsy punch when blunt.
- Place the discs from each sample in separate 2 mL centrifuge tubes (Figure 5). A total of 18 discs are recommended for each tube.

- Add 1 mL of hexane (previously decanted from stock bottle to Schott bottle) to the tube (Figures 6 and 7).

Step 4. Incubate Samples

- Close the centrifuge tubes and incubate at room temperature for 10 minutes. Mix the sample solution using the vortex mixer at the start and end of the 10 minutes.
- Turn on the spectrophotometer to allow the lamp to warm up (or as recommended by the manufacturer).

Step 5. Load and Dilute Samples

- Invert sample three (3) times to mix solution.
- Add sample volume to small volume quartz cuvette (Figure 8). The cuvette is fragile and expensive. Be careful when handling.



Figure 1. Peeling apple from stem bowl to calyx. (Photo: D. Rudell)



Figure 2. Segments of peel from three apples. (Photo: D. Rudell)



Figure 3. Remove discs of peel with a biopsy punch. (Photo: D. Rudell)

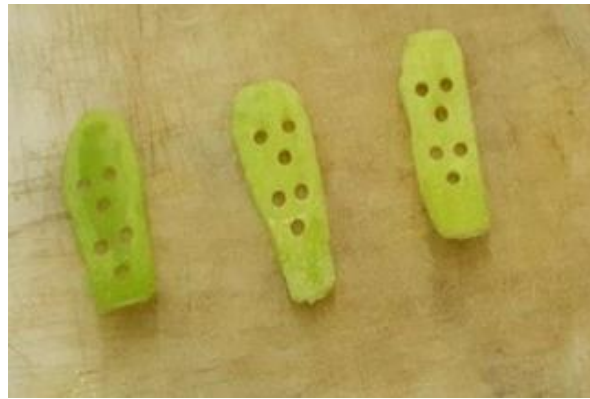


Figure 4. Six peel discs were removed from each segment of peel. (Photo: D. Rudell)



Figure 5. Eighteen discs in a centrifuge tube. (Photo: D.Rudell)



Figure 6. Remove 1 ml aliquot of hexane. The hexane should be decanted from stock bottle into a Schott bottle to prevent contamination. (Photo: D. Rudell)



Figure 7. Add 1 mL hexane to the centrifuge tube with discs of peel. (Photo: D. Rudell)



Figure 8. Add sample solution to quartz cuvette, after 10 minute incubation and triple inversion. (Photo: D. Rudell)

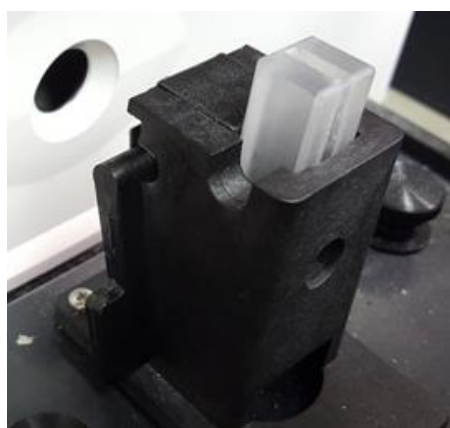


Figure 9. Read sample absorbance on spectrophotometer, after blanking with hexane. (Photo: D. Rudell)

The quantification of compounds using spectroscopy relies on the Beer-Lambert Law. The law is not accurate beyond the linear range of the instruments (i.e., very high or low concentrations), so the extract in this assay needs to be diluted with hexane to obtain an accurate reading. The absorbance at 232 nm is a useful indicator for this assay.

Step 6. Record Spectra

- Zero the spectrophotometer using hexane.
- Place sample cuvette into holder and lock (Figure 9).
- If necessary, dilute with hexane, depending on the linear range of the spectrophotometer provided by the manufacturer.
- Record the dilution volume.
- Record the absorbance vs. wavelength at 281 nm.

Step 7. Calculation of Conjugated Trienols

Further details on the calculations are provided below, and [an Excel spreadsheet template](#)¹ is also available for use. Note α -farnesene can also be calculated using this assay, if desired.

- Calculate the total disc surface area (S) using Equation 1.
- Calculate the dilution factor (D) using Equation 2.
- Calculate concentration of conjugated trienols using Equations 3 and 4. Equation 4 calculates the average value of the replications.

Equations and an example calculation are in the Example Calculation section.

Step 8. Interpretation & Decision-Making

- Rank the samples from highest to lowest CT values. Decision guidelines are given below.

The assay can be used to predict the relative risk of scald development among different rooms. Our evidence suggests it has not been effective for ranking among orchards within the same room given the same crop protectant (DPA and/or 1-MCP). The CA atmosphere is the critical factor for scald control – especially for organic production.

A room containing fruit with higher concentrations of conjugated trienols (compounds that the assay measures) compared to other rooms should be regarded as higher risk, and may indicate that storage conditions and/or crop protectant regimes are sub-optimal for scald control.

The best results are obtained by tracking levels of conjugated trienols over a 12-week period starting at harvest. This will indicate whether the storage regime is controlling scald. Rooms where values are increasing dramatically within the first 3 months are at high risk. CT levels increase very little if crop protectants and/or CA environment is controlling scald. As a guideline, we cautiously suggest (for ‘Granny Smith’) values of >10 nmol/cm². Within the first 3 months indicate imminent scald development upon removal from CA storage – and possibly in CA storage – as the CT values continue to increase.

Knowledgeable examination and interpretation of the results of your analysis is critical to making correct storage decisions. Be careful not to draw conclusions beyond the limits of the information provided by this assay.

¹ Download link: <http://extension.wsu.edu/publications/wp-content/uploads/sites/54/2017/10/FS287E-Calculator.xlsx>

Step 9. Disposal

Hexane is an organic solvent and should be disposed of correctly. Empty used centrifuge tubes into a disposal bottle until full. Contact a waste disposal company for assistance.

Equipment

Spectrophotometer Options

There are many UV-Vis spectrophotometers available. Considering the value of the fruit in storage that would be tested, it is worth purchasing a more precise spectrophotometer at a slightly higher marginal cost.

If you are considering using the spectrophotometer for other assays, we suggest buying an instrument with higher specifications, two examples are the Agilent Cary 60 or Shimadzu UV-1800. If cost is prohibitive, there is a suitable number of mid-range instruments available such as the Thermo Fisher Genesys 10S UV-Vis (Table 1). There are less expensive instruments with lower specifications, but this assay has not been tested on these.

Pipette options

Positive displacement pipettes are preferred to an air displacement pipette to improve accuracy with small volumes. Two variable volume pipettes are recommended for this assay, one accurate for approximately 100 μL and another for 1,000 μL . These cost approximately \$350 each and can be ordered online.

Vortex mixer options

The vortex mixer is not essential for this assay but does improve mixing and extraction of the conjugated trienols. It can be bought online for \$100-\$150.

Micro-Centrifuge Tube and Rack Options

Use air-tight tubes with rubber gaskets. These can be bought online in packs of 500 for approximately \$55. A rack can be bought from the same supplier.

Biopsy punch

A range of biopsy punches is available at online retailers and laboratory supply companies. The approximate cost is \$1.20 per punch.

Example Calculation

The symbols for the variables and constants used, and which equations they are used in, are given in Tables 2 and 3. If you require assistance or clarification with any step in this assay, please contact the authors.

Table 1. Summary specification comparison between three UV-Vis spectrophotometers.

Instrument	Spectral Bandwidth	Wavelength Accuracy	Reproducibility
<i>Agilent Cary 60</i>	±1.5 nm	±0.06 nm @ 541.94 nm	±0.01 nm
<i>Shimadzu UV-1800</i>	1 nm	±0.1 nm @ 656.1 nm ±0.3 nm over range	±0.1 nm
<i>Thermo Fisher Genesys 10S UV-Vis</i>	1.8 nm	±1 nm	±0.5 nm

Table 2. The symbols, description of variables used in this assay, and which equations where these are used.

Variable	Description	Equation
S	Total disc surface area (cm ²)	1, 3
f	Number of fruit in replication	1
n	Number of peel discs per fruit in replication	1
d	Diameter of peel disc (mm)	1
s	Volume of sample solution in cuvette (mL)	2
h	Volume of hexane used to dilute sample (mL)	2
CT_{rep}	Concentration of conjugated trienols in one replication (nmol/cm ² peel)	3
A_{Δ}	Difference between A_{281} and A_{290}	3
A_{281}	Absorbance at 281 nm	3
A_{290}	Absorbance at 290 nm	3
D	Dilution factor	3
CT_{sample}	Concentration of conjugated trienols in one sample (nmol/cm ² peel)	4
i	Number of a single replication in a sample	4
r	Number of replications per sample	3

Table 3: The symbols, description and values of constants used in this assay, and which equations where these are used.

Constant	Description	Value	Equation
CF_1	Conversion factor: mm ² to cm ²	100	1
V	Volume (L)	0.001	3
CF_2	Conversion factor: mol to nmol	1,000,000,000	3
ϵ	Extinction coefficient: conjugated trienol (M ⁻¹ .cm ⁻¹)	25,000	3
L	Cuvetter length (cm)	1	3

Equation 1.
$$S = \frac{f \cdot n \cdot \pi \cdot (d/2)^2}{CF_1}$$

Equation 2.
$$D = \frac{s}{s + h}$$

Equation 3.
$$CT_{rep} = \frac{A_{\Delta} \cdot D \cdot V \cdot CF_2}{\epsilon \cdot L \cdot S}$$

Equation 4.
$$CT_{sample} = \sum_{i=1}^r \frac{CT_i}{r}$$

Equation 1: The total disc surface area (S) is 2.262 cm² for 3 fruit per replication (f) and 6 punch holes per peel segment (n), with a 4 mm disc diameter (d).

Equation 2: The dilution factor (D) for the 3 replications of this sample was 0.75, with 0.75 mL of sample (s) and 0.25 mL of hexane (h).

Equations 3: This value is calculated for each replication in the sample which is 3 in this example. The concentration of the conjugated trienols in these 3 replications were 1.64, 2.39, and 2.05 nmol/cm² (CT_{rep1}, CT_{rep2}, and CT_{rep3}). D, V, CF₂, ε, and L are constants available in Table 3; S was calculated in Equation 1.

Equation 4: This equation simply calculates the average value of the 3 replications (r) in Equation 3. CT_{sample} = 2.03 nmol/cm² with a standard deviation of 0.38 nmol/cm². This needs to be determined for each sample.

These values would then be consolidated into a table with the other samples to determine the relative risk of scald development.

Equation Variable Value

1	S	2.262 cm ²
	f	3
	n	6
	d	4 mm

Equation Variable Value (Rep 1) Value (Rep 2) Value (Rep 3)

2	D	0.75	0.75	0.75
	s	0.75 mL	0.75 mL	0.75 mL
	h	0.25 mL	0.25 mL	0.25 mL

Equation Variable Value (Rep 1) Value (Rep 2) Value (Rep 3)

3	CT _{rep}	1.64 nmol/cm ²	2.39 nmol/cm ²	2.05 nmol/cm ²
	A _Δ	0.1233	0.0996	0.0738
	A ₂₈₁	0.3413	0.4543	0.2738
	A ₂₉₀	0.2180	0.3547	0.2000

Equation Variable Value

4	CT _{sample}	2.03 nmol/cm² (Standard Deviation = 0.38)
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