Phenotyping in Peach

by

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List of traits to phenotype

- Bloom time & type   Slide 4-5
- Leaf gland type?   Slide 6
- Maturity date   Slide 7
- Crop load rating ? Still missing procedure   Slide X
- Yield rating ? Still missing procedure   Slide X
- Fruit   Slides 9-32
  - type & fuzz
  - size (weight?), fruit tip
  - Base color
  - Flesh color
  - Freestone/clingstone
  - Firmness by FTA or penetrometer
  - pH and TA
  - SSC (°Brix)
  - Flesh browning, texture and bleeding (?)
  - Enzymatic browning and Pehnolic levels (?)
- Pit (size - weight)   Slide 33
- Split pit & pit fragments   Slide 34

General remarks

- All measuring units in metric system
- Score fuzz in the field
- If fruit collected in plastic bags remove as soon as possible and place in open container
- *Data must be as objective as possible if our pooled data is to have a chance at finding high correlations with later QTLs, so things like ripe date need to be standardized*

Bloom date & type

- Bloom date is taken at the same time every day as full bloom when 60-80% of the flowers are open.

Showy

Non showy

Leaf gland type?

Reniform

Globose

Eglandular

Maturity Date

- Maturity date will be the recorded date when fruit are first harvested.
- Fruit should be harvested and consequently analyzed at tree ripe stage.
- To ensure uniformity of data collected at the tree ripe stage for all peach germplasm:
- *Wait until a very few fruit are soft/edible (whatever that is for that type peach) then collect the fruit less ripe as 'tree ripe' record the harvest date and proceed with data collection.*

Fruit Collection Procedure for Fruit and Pit Phenotyping

- Collection of fruit once per season when fruit deemed 'tree ripe'.

- Approximately **20** (5 -10 in seedlings) fruits harvested for phenotyping, additional fruit might be needed if enzymatic browning and phenolic content are measured

- Prior to evaluation the fruit should be put in a cardboard or plastic box/container labeled with the genotype and harvest date to allow fruit to "breathe" and dry out if there is excess moisture from dew/rain

Fruit set – Byrne scale

- 0=none

- 5=full crop, 6-8" (15-20cm) spacing between fruit

- 7=2x fruit needed, 3" (7.5cm) spacing

- 9=4x fruit, 1" (2.5cm) spacing

- This is the scale I have used in previous studies and during my cultivar/selection evaluations.

Fruit quality traits

- Select 5 fruits to measure fruit quality traits

- Place 5 fruits in a fruit tray designated areas labeled 1 -5.

- Make sure that the fruit and its parts are always kept in the same order.

Fruit type & fuzz

**Fruit type:**   **Peach (1)**   **Nectarine (0)**

Fruit fuzz:

- Measure qualitatively using following scale:
  0 – none
  3 – very slight, can eat without rubbing fuzz
  5 – medium, typical of many modern cultivars
  7 – heavy, like some old cultivars no fun to eat off tree

Fruit size & fruit tip

- Measure fruit size (diameter) across suture and across cheek area and fruit length by a micrometer caliper.

Optional – Fruit tip or fruit beak:
-measure distance beak
extends from fruit circle?
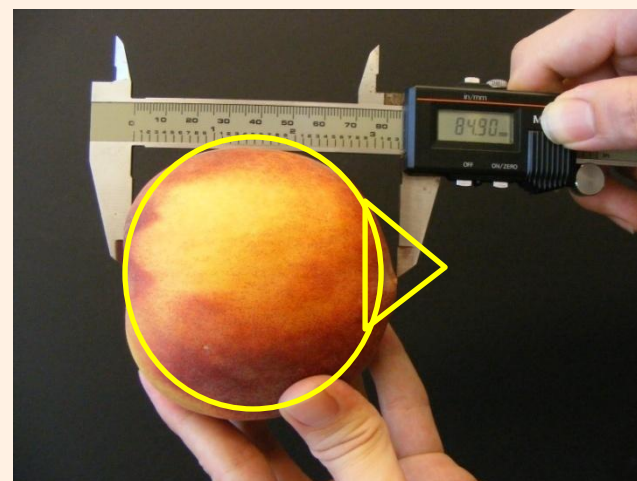
Fruit weight & Base color

- Measure all 5 fruits together and record average weight

- Visually estimate % blush as:

  0 – no blush; **1** - 1-20%; **2** - 21-50%; **3** - 51-80%; **4** – 81-99%; **5** - 100%

- Color is measured with a standard Minolta chroma meter as CIE L*a*b*

- Measure darkest and lightest (non-green) epidermis sections on both cheeks (red blush over base color will complicate this: perhaps take reading on darkest non-blush (yellow or white) section].

- Measure flesh color after removing 1 cm skin/flesh

Flesh Color

- Determine base layer color
    - white/yellow/orange – visually and
    - quantified by earlier Minolta data

- Then rate proportion of red overlay (%), as:
  0 – no red overlay; **1** - 1-20%; **2** - 21-50%;
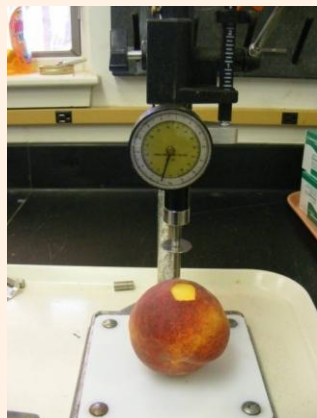 **3** - 51-80%; **4** – 81-99%; **5** - 100%

Fruit Firmness

Fruit firmness can be measured two ways:

1. Using Fruit Texture Analyzer:
2. Using mounted or hand held penetrometer

If mounted or hand held penetrometer were to be used, to assure consistency with FTA measurement

– first measure skin,

– then outer flesh (after removing 1 cm skin), and

– inner flesh (measure from inner pit cavity)

Fruit Texture Analyzer (FTA)

1. Turn on the Fruit Texture Analyzer (FTA) by pressing the switch next to the power cord connection at the back of the FTA.
2. Turn on the computer.
3. Double click the 'FTAWin' icon on the desktop to open the FTA control program.
4. Click the 'Start Test' button.
5. Select the type of fruit to be tested from the 'Profile' drop down list.

FTA continued

7. Select the number of samples and type of variety under the 'Parameters' heading

8. Select the supplier and enter a reference number and date under the 'Test Identification' heading.

9. Click 'OK' at the top of the window.

10. Place the fruit on the three legged fruit holder and center below the FTA probe

FTA continued

11. Press and hold the green button on the front of the FTA to make a measurement. If the fruit needs repositioning, releasing the button will stop the probe from lowering. Once properly positioned, press and hold the button to continue the measurement process. Once contact is made with the fruit, the probe will decrease in speed until the set depth is reached. Once the probe begins to rise, the button can be released and the probe will return to the highest position.

12. To make the next measurement, repeat step 10.

13. The location of the data file can be found at the bottom of the program window.

14. Go to step 4 to perform another test run.

Fruit Firmness by penetrometer
use 8mm flat tip probe

To be consistent with FTA: measure skin, then outer flesh (after removing 1 cm skin), and then inner flesh (measure from inner pit cavity)

1. Shave skin to a standard depth of 1cm from both cheeks of peach fruit (use fruits prepared for flesh color measurement)
2. Position fruit with prepared area under the penetrometer probe
3. Make sure that the scale is in 0 position
4. Press penetrometer into the fruit until the marking on the probes
5. Record the force
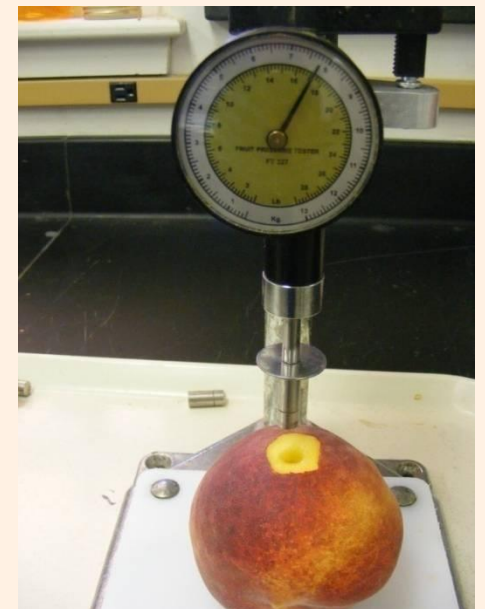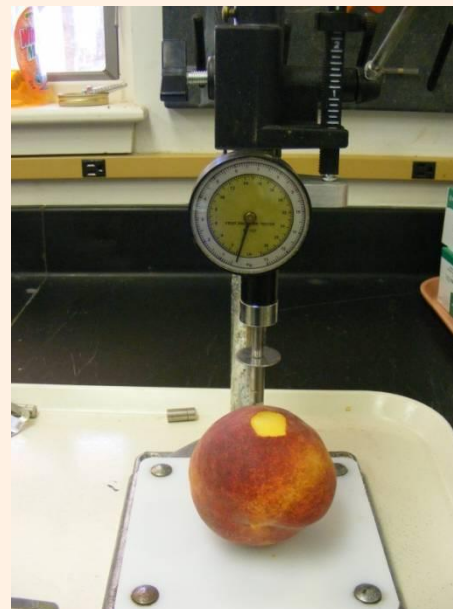
pH & TA

I. Materials

A. Required: pH meter or phenolphthalein, burette, burette clamp and stand, gram scale, graduated cylinder, beakers, 0.1N NaOH solution

B. Optional: magnetic stirrer & stir bar, automatic titrator

pH & TA Procedure

A. Obtain at least 50 ml of clear juice by one of the following methods:

1. Cut 1-2 longitudinal slices from each fruit (5 fruits total), press with a hand press, and filter through cheesecloth, or

2. Cut fruit into a blender, homogenize, centrifuge slurry, and pour off clear liquid for analysis.

**   Sugar levels often vary within the fruit, being higher at the stem-end and lower at the calyx-end. For this reason, it is important to use longitudinal slices of fruit (from end to end) when sampling.

pH & TA Procedure

B. Make sure samples are at room temperature before taking measurements.

C. Measure the pH of the samples with a pH meter and record the value.

D. For each sample, weigh out 6 grams of juice into a 100 ml beaker.

E. To each sample, add 50 mls of water.
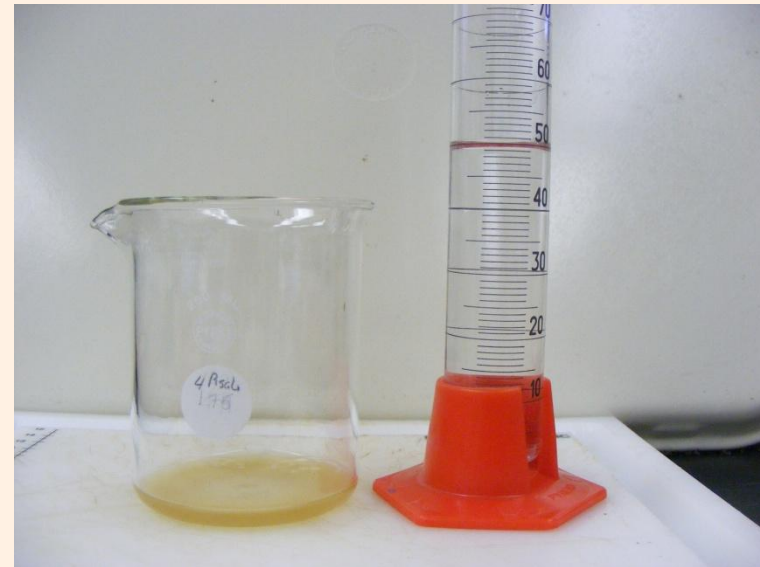
pH & TA Procedure

F. Titrate each sample with 0.1 N NaOH to an end point of 8.2 (measured with the pH meter or phenolphthalein indicator) and record the milliliters (mls) of NaOH used.

Optional – automatic titration unit can also be used

G. Calculate the titratable acidity using the following formula:

% acid = [mls NaOH used] x [0.1 N NaOH] x [milliequivalent factor] x [100]
grams of sample

Soluble solids (SSC)

I. Theory

A. Sugars are the major soluble solids in fruit juice. Other soluble materials include organic and amino acids, soluble pectins, etc. Soluble solids concentration (SSC%, °Brix) can be determined in a small sample of fruit juice using a hand held refractometer. This instrument measures the refractive index, which indicates how much a light beam is "bent" when it passes through the fruit juice.

B. Temperature of the juice is a very important factor in the accuracy of reading. All materials expand when heated and become less dense. For a sugar solution, the change is about 0.5% sugar for every 10°F. Good quality refractometers have a temperature compensation capability.

SSC continued

A. Extract clear juice from fruit to be sampled.

   1. Sugar levels often vary within the fruit, being higher at the stem-end and lower at the calyx-end. For this reason, it is important to use longitudinal slices of fruit (from end to end) when sampling. Use few drops from juice extracted for pH and TA

B. Place a drop of juice on refractometer prism.

C. Lower cover plate and read.

   1. In juice samples with a high starch content, like unripe kiwi, it may be difficult to read the refractometer because the starch settles out on the prism. To remedy this, put your thumb on the cover plate, turn the refractometer upside down, and re-read. This way the starch settles out on the cover plate and does not blur the reading.

D. Rinse prism between samples with distilled water and dry with a soft, lint-free tissue (Kimwipe).

SSC continued

Refractometer maintenance and calibration

A. Clean the instrument after each use with distilled water. Dry with a soft tissue (Kimwipe).

B. Calibrate with a drop of distilled water. Adjust reading to 0 °Brix if necessary with the small set-screw on the back. Verify accuracy with a drop of 5 or 10% sucrose solution (5 grams sugar in 100 mls of distilled water).

C. Do not submerge the refractometer when cleaning. If water gets into the instrument it will need to be sent out for repair and resealing.

Stone Fruit Internal Breakdown Evaluation

Symptoms of stone fruit internal breakdown (IB) or chilling injury (CI) include:

• browning of the flesh,

• development of a mealy or leathery texture,

• accumulation of red pigment in the flesh, and development of off-flavors.

• These symptoms can be measured as follows: How long to store and what temp?

Flesh browning

- Measured qualitatively on a scale from 1-6

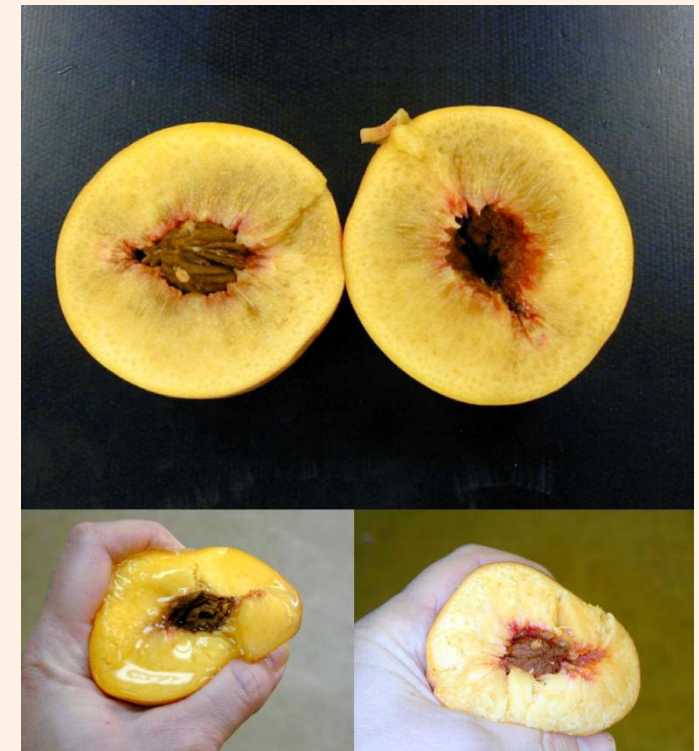| Score	Description
1	None
2	Very slight browning in the pit cavity
3	Slight browning in the pit cavity and surrounding tissue
4	Moderate browning on less than 50% of the flesh
5	Severe browning on 50-75% of the flesh
6	Extreme browning covering most of the flesh
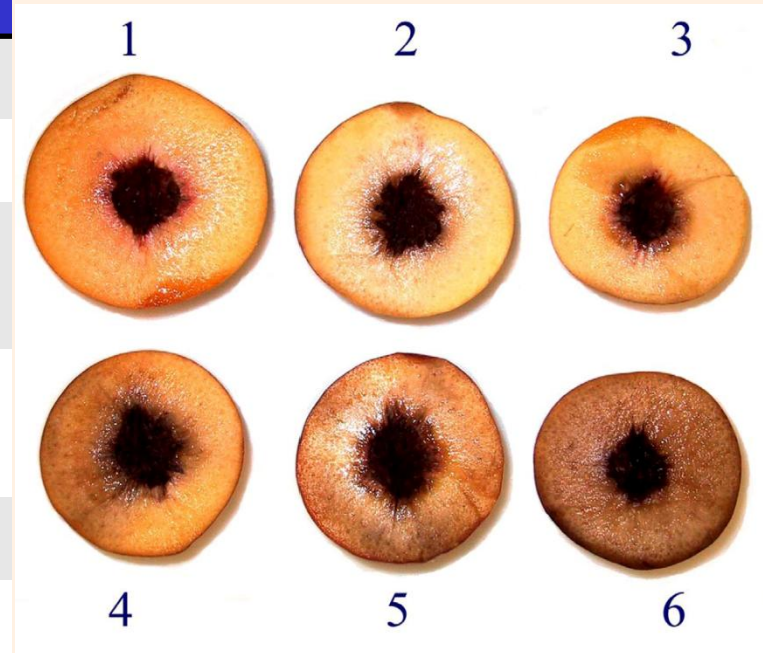
Peach flesh browning scale

Flesh texture

- Measured qualitatively on a scale from 1-3

Score	Description
1	Juicy fruit
2	Moderately leathery or mealy fruit (small amount of juice released upon squeezed)
3	Severely leathery or mealy fruit (almost no juice released upon squeezing)

Peach flesh browning (top), juicy fruit (bottom left), and mealy fruit (bottom right).

Flesh bleeding

- Measured qualitatively on a scale from 1-3

Score	Description
1	No bleeding
2	Moderately bleeding with red pigment covering less than 50% of the flesh
3	Severe bleeding with red pigment covering more than 50% of the flesh

# Estimating Enzymatic browning

Bruise damage generated by a standardized impact was used to assess enzymatic browning potential.

• Test 5 fully ripe fruit for bruising by dropping 2.5cm diameter (64.2g) steel ball from a 30 cm height onto the more mature peach fruit cheek. Mark the impact area with black indelible ink.

• Place fruit in cupped plastic trays and hold at 22°C for 24h. After 24h rate flesh bruise color at a 6mm depth using standardized color chart developed according to the following scale:

1- no bruise visible, 2 - translucent area with no discoloration, 3 - pale tan bruise, 4 - light brown bruise, 5 - brown bruise

Estimating phenolic levels

• Test 5 fully ripe fruit , use slice from firmness measure

Use Nitroso rapid assay (Kader and Chordas, 1984).

1. First slice fruit parallel to the suture to a depth of 6mm.

2. Apply one drop of each of the following reagents in succession to the fruit flesh at the approximate center of the 4-5 cm of exposed tissue from slice:

- sodium nitrate (10%)

- urea (20%)

- acetic acid (10%)

3. After 4 minutes add 2 drops of sodium hydroxide (8%) and rate resulting cherry red color on a 1-5 scale using a standardized color chart (Kader and Chordas, 1984).

Estimating phenolic levels – color chart (Rating scale)
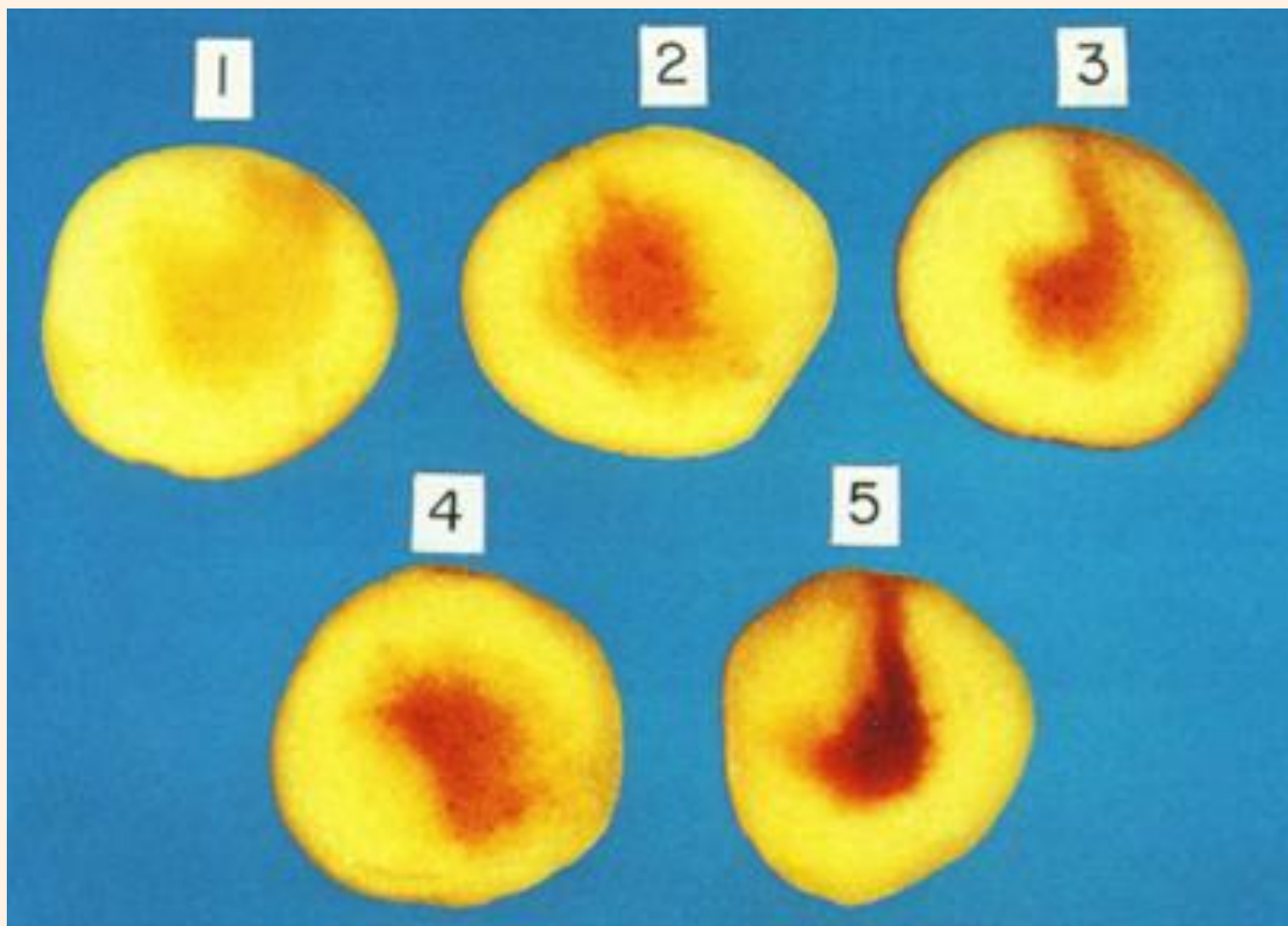
PPO activity. Dark cherry-red color develops in a nitroso test, indicating phenolics content in fruit. Combined scores measure browning potential
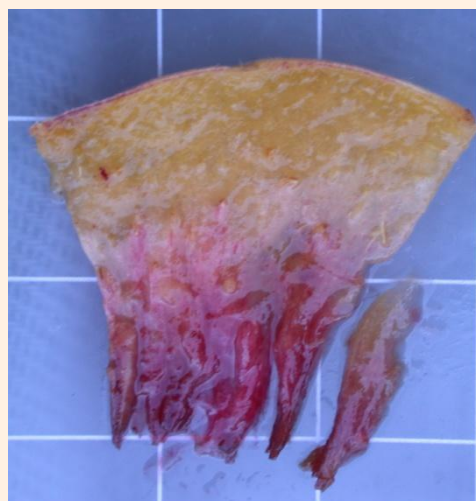
Freestone/ clingstone

- Measure qualitatively on a scale 1-3

| Score	Description
1	Freestone – flesh and pit completely separate
2	Semi clingstone – flesh partially separates from pit - test in soft fruit since many semi clingstone fruit become freestone when soft
3	Clingstone – no separation between flesh and pit

Melting   /   Nonmelting

Pit measurements

Use pits from 5 fruits used for fruit quality measurements

• Weigh all 5 pits for average mass if fruit mass was recorded or

• Measure pit tip length (in mm)

• Measure pit dimensions (in mm) using micro caliper
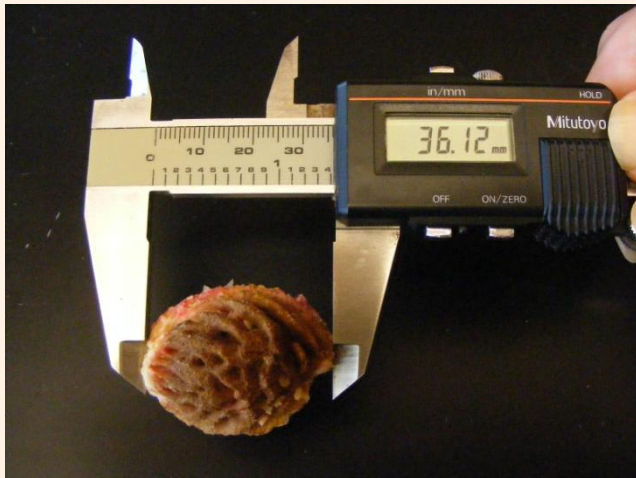
Split pits & pit fragments

- Split pits simply rated as proportion of split pits/normal pits.

- Pit fragments is rated as average number of pit fragments (> 1 cm) in fruit pit cavity after halving. *(number of easily measured large fragments should be good indication of preponderance small fragments as well).*