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Developing a quantitative method to evaluate peach (*Prunus persica*) flesh mealiness

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Abstract

A quantitative method to measure peach flesh mealiness based on free juice was developed. Free juice was measured by subjecting fruit tissue to a pressing force of 667 N for 1 min before centrifugation. After this extraction, the juice was collected and centrifuged at 6000*g* for 10 min. The supernatant was weighed and used to represent the free juice from the total initial tissue. The percentage of free juice was more sensitive and it had a higher correlation to mealiness measured visually and by a trained panel than the extractable juice method. Our work also demonstrated that trained judges were able to perceive mealiness earlier than visual mealiness symptoms were expressed. This free juice measurement provides an objective and accurate method to evaluate mealiness potential of stone fruit breeding lines and fruit biochemical and/or physiological changes during postharvest storage.

Keywords: Chilling injury; Internal breakdown; Free juice; Extractable juice; Apparent juice; Expressible juice; Sensory evaluation; Stone fruit

1. Introduction

Some apricot, nectarine, peach, and plum cultivars develop chilling injury (CI) symptoms during low temperature storage and/or shipping. CI is a physiological disorder whose symptoms are expressed during ripening under non-stressed conditions (Ben Arie and Lavee, 1971). Chilled damaged fruit develop flesh mealiness (FM), a dry or mealy flesh texture with a grainy sand-like texture, flesh browning (FB), 'off flavors', and fail to ripen properly during prolonged cold storage and/or after ripening at room temperature. FM is also named woolliness, especially by researchers from South Africa (Von Mollendorff et al., 1992a,b). These CI symptoms are also known as internal breakdown (IB) (Luza et al., 1992; Smith, 1934). Because these symptoms reduce consumer acceptance, their onset limits a fruit's potential postharvest market life (Crisosto et al., 1999). Peach susceptibility to CI varies according to genetic background (Anderson, 1979; Crisosto et

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al., 1999), maturity (Von Mollendorff, 1987), and orchard factors (Crisosto et al., 1997).

In our previous work (Crisosto et al., 1999), most of the CI-susceptible cultivars grown in California developed FM prior to FB symptoms; only rarely did FM and FB develop at the same time. Several cultivars developed FM without FB symptoms. Thus, development of postharvest handling treatments or breeding programs to reduce CI problems should be focused on FM rather than FB. A rapid evaluation of FM symptoms is usually a subjective visual determination. The measurement of the most obvious characteristic of the FM problem, decreased fruit juice, can be used as a more objective assessment of FM. Approaches for this analysis have been previously described (Lill and van der Mespel, 1988; Von Mollendorff et al., 1992a,b). Nevertheless, there is still the need for a rapid, sensitive and quantitative method to measure the onset of FM.

The roles of polygalacturonase (PG) and pectinesterase (PE) activities on pectin degradation during FM development have been well documented (Ben Arie and Lavee, 1971; Ben Arie and Sonego, 1980; Von Mollendorff and De Villiers, 1988b; Dawson et al., 1992; Lurie et al., 1994; Zhou et al., 1999). Pectins from sound peaches and nectarines are normally poor gel formers due to the presence of acetyl groups, high methoxyl levels, and relatively low molecular masses. The fact that cold storage below 8 °C reduced fruit production and/or activity of PG but did not significantly affect PE activity (Ben Arie and Lavee, 1971; Ben Arie and Sonego, 1980) led to the assumption that the altered balance of PG and PE activities at low temperatures or during ripening lead to incomplete or unbalanced cell wall pectin degradation. Pectins with longer polymer backbones and reduced methyl ester content have a high gel formation capacity. Available water and solutes (primary sugars) from surrounding tissues are trapped as apoplast conditions trigger pectin gel formation, inducing a dry mealy fruit texture (Ben Arie and Lavee, 1971; Buescher and Furmanki, 1978; Zhou et al., 1999). The water in fruit can be classified as 'free' or 'bound' water. Free water is the water that gives ripe fruit a juicy character; and bound water is that which is associated with polymer or solute hydration spheres, and pectin gels in mealy fruit. Thus, the total water content in both mealy and juicy fruit can be similar (Von Mollendorff and De Villiers, 1988a), but fruit texture can differ substantially if the proportions of free and bound water are altered during the gel formation.

Based on this concept, quantitative methods for mealiness assessment, using the supernatant after centrifuging of homogenized fruit tissue, were developed by Lill and van der Mespel (1988) and Von Mollendorff et al. (1992a,b). In the method of Von Mollendorff et al. (1992a,b), fruit tissue was homogenized for 60 s in a Waring blender before centrifugation for 10 min at 1000*g* (supernatant liquid was defined as 'extractable juice'). In the 'apparent juice' method (Lill and van der Mespel, 1988), fruit tissue was gently disrupted by placing it in a syringe and extruding it by forcing it through a luer hub. After disruption, collected tissue was centrifuged at 12 000*g* for 10 min. In most cases it was difficult to force tissue through the luer hub and also to separate the gel phase from the liquid phase. We and others have observed, in some cases, that gel former pectins can be created during juice disruption and/or centrifugation, producing additional undesired gel formation. In both of the methods, the volume of the supernatant is recorded as a percentage of the initial tissue mass. This extractable juice quantitative method has been used to report FM by several researchers; however; some contradictions are found in the literature. In some work with peaches and nectarines, the extractable juice percentage did not correlate well with visual mealiness symptoms. For example, in 'Flavortop' nectarine, during 4 weeks storage at −0.5 °C, the extractable juice remained constant at approximately 50 and 30% for mature and ripe fruits, respectively. During this same storage period, visual mealiness of ripe fruit increased from none detectable to 80% (Von Mollendorff et al., 1992a). In Israel, preharvest application of gibberellic acid significantly delayed visual mealiness development in nectarines without affecting their apparent or expressible juice contents (Zilkah et al., 1997). In these cases, woolliness was evaluated visually and by hand squeezing the fruit to collect juice. These reports suggest that the extractable juice method may not be a reliable indicator of FM.

The research described in this paper demonstrates that free juice assessed after a pressing extraction prior to centrifugation is highly related to mealiness development and can be used as a quantitative indicator of FM.

2. Materials and methods

'Rich Lady', 'Elegant Lady', 'Zee Lady' and 'O'Henry' peach and 'Summer Grand' nectarine cultivars commercially grown in California and Chile were tested for CI symptoms that developed after fruits were stored at 0 or 5 °C and then ripened. Fruit samples (100 fruits) of each cultivar were collected at the California well-mature stage according to the California Tree Fruit Agreement ground-color chips from each of the three trees (replications) growing at the Kearney Agricultural Center (KAC), or from the commercial orchards near the KAC. Fruits were forced-air cooled to 0–2 °C within 12 h of harvest and then stored at either 0 or 5 °C (with 90% relative humidity) for up to 3 weeks. Chilean tree fruits were collected upon arrival at Long Beach, CA after shipment at 0 °C lasting approximately 14 days.

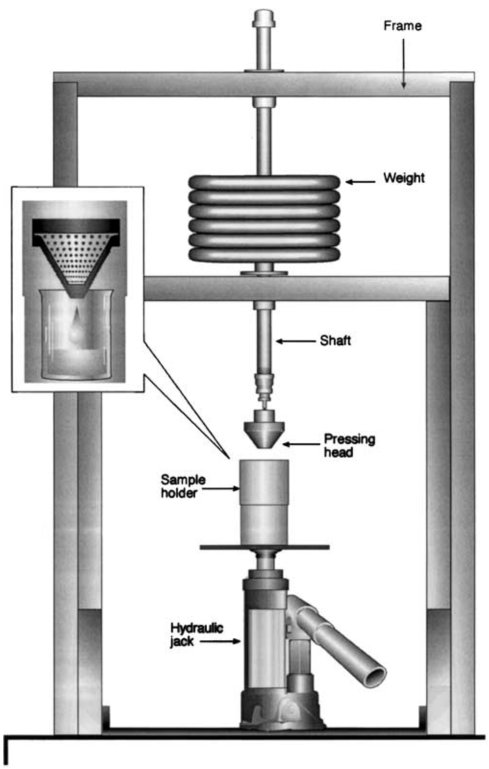

Fig. 1. Scheme of the pressing apparatus.

Three replications of ten fruit samples each were removed weekly from storage (0 or 5 °C), ripened at 20 °C until firmness reached between 10 and 18 N (measured with a UC firmness tester, 7.9-mm tip), then evaluated for CI symptoms. The ripening period varied from 3 to 7 days depending on the fruit's initial firmness and its rate of softening. Flesh mealiness and flesh browning symptoms were visually determined immediately after cutting the fruit in half according to Crisosto et al. (1999). Fruits with little or no juice after hand squeezing were considered mealy.

2.1. Free juice measurements

To evaluate the effects of CI development on the amount of free juice released by this method, 'O'Henry' and 'Elegant Lady' peaches from California and Chile with different degrees of mealiness (visually assessed) were used. Peaches were ripened at 20 °C until soft (firmness 10–18 N) measured with a UC firmness tester, 7.9-mm tip. Forty grams of fruit tissue from each fruit/replication was used. To obtain the tissue, a wedge was cut longitudinally from the fruit with an approximate width of 25 mm at the back. This wedge was then cut into three sections and wrapped with four layers of cheesecloth (20 × 20 cm) before being compressed with a self-made press. The press (Fig. 1) consisted of a wooden test frame supporting a shaft. On the top of this central shaft was a fixed weight (load) and on the bottom was a pressing head. The pressing head (frustum of a right circular cone) measured 25 mm in diameter on the bottom (fruit contact area), 70 mm in diameter at the top, and had a height of 45 mm. The fruit tissue sample was placed in a sample holder inside a perforated steel basket. The perforations in the basket allowed the expressed juice to pass through a funnel into a collection beaker at the base of the sample holder. The sample holder was raised hydraulically onto the pressing head, raising it until the load was released from the resting position. The use of this self-made press assured the application of a constant load on the tissue over time which is not possible using our Instron model. After pressing the tissue for 1 min, the sample holder was lowered. Then, the tissue was repositioned in the holder and pressed again. Fruit tissues were exposed to different pressing forces (from 222 to 890 N), for different durations (from 1 to 10 min), and different repetitions of pressing (from 1 to 6). After this, the extracted juice was centrifuged at 6000*g* for 10 min and the supernatant weight was used to determine the percentage free juice based on the initial fruit tissue sample weight.

The percentage 'extracted juice' was measured according to Von Mollendorff and De Villiers (1988a). In this method, fruit tissue (1.5*g*) was homogenized with a blender for 1 min and allowed to stand for 15 min prior to centrifugation at 6000*g* for 10 min. The supernatant weight (extracted juice) was used to determine the percentage extractable juice based on the initial fruit tissue sample weight.

2.2. Free juice method sensitivity

To compare the relationships between free juice, extractable juice, and mealiness incidence, 'Flavorcrest' and 'Elegant Lady' peaches with different degrees of mealy fruits were used. Sixty mealy and 60 juicy peaches were chosen from the different storage temperatures. These mealy and juicy fruits were cut into 15 g portions. Then, by combining varying weights of the mealy and juicy fruit portions, different fruit mealiness percentages were created. Free juice and extractable juice measurements were determined in the range of different mealiness percentages artificially created as previously described.

2.3. Sensory evaluation

To evaluate the sensory perception of mealiness (the feeling of graininess, like sand) a trained panel consisting of six screened judges was selected. Judges were screened and trained for their acuity in perceiving mealiness using a triangle test. In the triangle test, three samples of sliced mealy or sound peaches were randomly presented simultaneously, with two the same and one different. The respondent was asked to choose the odd sample. Six combinations were presented in random order to each potential judge. Using a one-tailed binomial test with a 0.05% significance level, only judges with five or six correct responses were used for the mealiness detection (O'Mahony, 1986). Each 'O'Henry' peach sample consisted of a slice cut from the bottom half of the peach with the skin removed. Each judge was instructed to cleanse his or her mouth with distilled water, chew the peach sample and mark a yes or a no on the scorecard for juiciness, mealiness and 'off flavor', then cleanse again before proceeding to the next sample. The parameters were defined as: juiciness = the feeling of juice freely flowing in the mouth; mealiness = the feeling of graininess, like sand; and 'off flavor' = not having the usual or standard peach flavor.

2.4. Statistical analysis

Data were subjected to ANOVA and LSD means separation at the 5% level on the free juice pressing force data. Correlation analysis was used for the analysis of data from the free juice sensitivity test and sensory evaluation data. The sensory evaluation data were also analyzed using population distribution comparison. The SAS statistical software (SAS Institute, Cary, NC) was used for these analyses.

3. Results and discussion

3.1. Free juice measurements

The percentage of free juice varied according to the pressing force that the fruit tissues were subjected to. In the three cultivars tested, free juice release increased as the pressing force increased, but it reached a plateau above 445 N (Fig. 2). There were no differences in free juice released from fruit tissues subjected to pressing forces between 445 and 890 N within each of the three

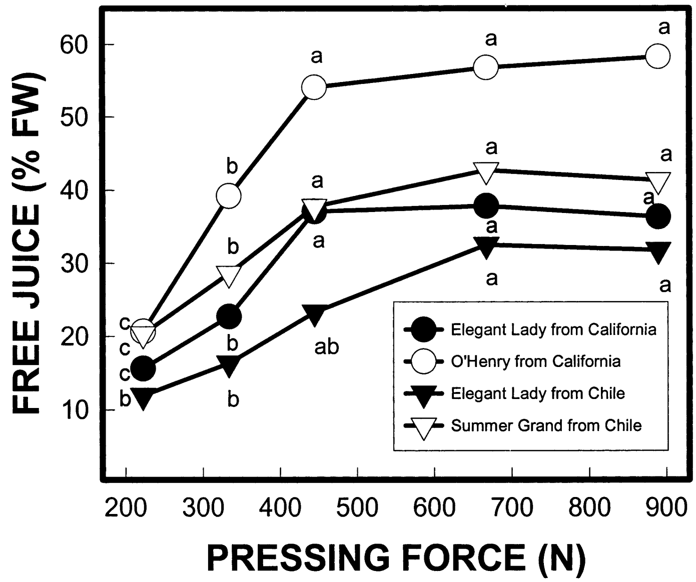

Fig. 2. Free juice released as a function of pressing force from peach and nectarine fruit. Different letters within each cultivar/origin represent significant differences at $P \leq 0.05$ by LSD means separation test.

cultivars tested. The percentage of free juice was highest for the California 'O'Henry' peaches, intermediate for the California 'Elegant Lady' peaches and Chilean 'Summer Grand' nectarines, and the lowest for the Chilean 'Elegant Lady' peaches. Both of the Chilean fruit samples were tested after shipment, approximately 21 days after harvest. The different percentages of free juice between cultivars indicated the current amount of chilling damage for each cultivar at that sampling date. Our previous laboratory evaluations of free juice for fresh ripe harvested peaches prior to storage yielded 68, 52, 59, and 57% for 'O'Henry', 'Rich Lady', 'Elegant Lady', and 'Zee Lady', respectively. The differences in the percentages of free juice released between these cultivars can be explained by the different cultivars' susceptibility to chilling injury, temperature conditions prior to collection, maturity at harvest, orchard factors during the growing season, storage period, and postharvest handling. The expression of CI symptoms such as lack of juice and FB are dependent on the genetic makeup and cold storage period (Crisosto et al., 1999). Probably CI-susceptible cultivars have differences in pectin structures and/or PG/PE balance that react in different ways to storage temperatures. The California 'O'Henry' peach had the highest percentage free juice because that fruit had a shorter exposure to low temperatures than the other three fruit samples. The percentage of free juice was the lowest in the Chilean 'Elegant Lady' because this cultivar was in cold storage for a longer period of time. 'Summer Grand' nectarine has a longer market life than 'Elegant Lady' peach when stored at 0 or 5 °C (Crisosto et al., 1999). This reported difference in potential market life between these two cultivars is consistent with the observation that the Chilean 'Elegant Lady' peach had a lower percentage free juice than the Chilean 'Summer Grand' nectarine even though they arrived on the same ship approximately 21 days after harvest. Even though fruits with different levels of chilling injury were used for this test, it is clear that the maximum amount of free juice will be released from fruit subjected to a pressing force between 445 and 700 N.

3.2. Free juice method sensitivity

There was a highly significant ($P \geq 0.001$) correlation between the range of artificially created percentages of FM fruit juice content and the measurements of free juice or extractable juice for 'Flavorcrest' and 'Elegant Lady'. It is important to point out that our sensitivity test is based on the assumption that mixing different proportions of mealy and juicy fruits will give the same results as fruit with different intensities of mealiness. Free juice and FM relationship ($R^2 = 0.91$) had a higher correlation than the relationship between the extractable juice and FM ($R^2 = 0.39\%$). Thus, changes in extractable juice only account for 39% of the variability in FM rather than 91%, as in the case of free juice. During our sensitivity test and/or cold storage time course, free juice values (low gel formation) were always higher than extractable juice values in the same fruit measurements. This suggests that more gel former pectins can be created during juice disruption during blending and/or centrifugation steps in the extractable juice method than in the free juice method. Thus, this additional undesired gel formation may be artificially increasing the amount of gel former pectins in the extractable juice method.

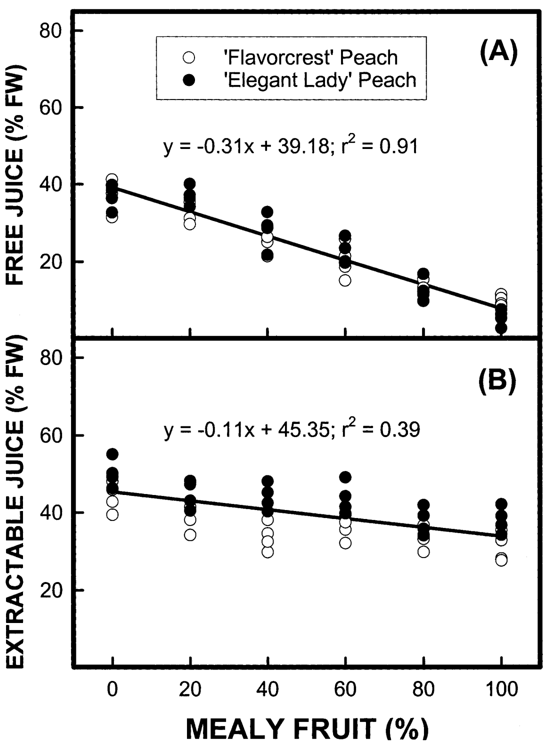

Fig. 3. Correlations between percentage mealiness artificially created in 'Elegant Lady' and 'Flavorcrest' peaches and (A) free juice and (B) extractable juice percentages.

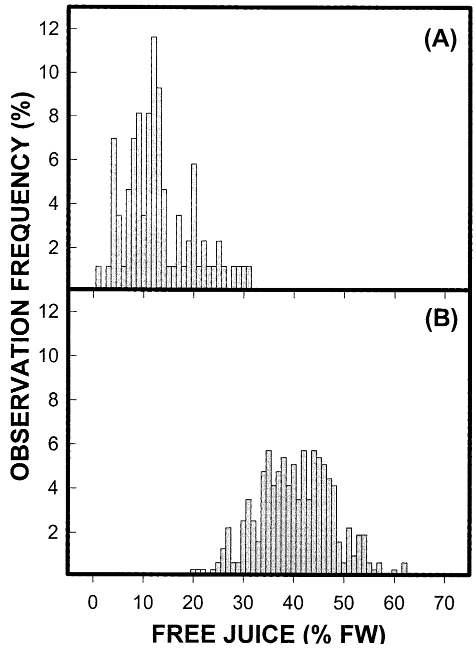

Fig. 4. Distribution of observed frequencies for 'O'Henry' peaches with different free juice percentages in the (A) mealy and (B) juicy categories according to our sensory trained panel.

A higher slope was consistently ($P \leq 0.001$) determined for the free juice and FM relationship than for the extractable juice and FM relationship (Fig. 3). This indicates that small changes in FM are more easily detected using the free juice method than the extractable juice method.

3.3. Sensory evaluation

Judges segregated 'O'Henry' peach samples into mealy and juicy categories. The free juice distribution in the mealy fruit population ranged from 2 to 32%, with a mean of 16% and a standard deviation of 9.8% (Fig. 4A). The percentage of free juice in the juicy fruit population ranged from 20 to 62% with a mean of 40% and a standard deviation of 8.4% (Fig. 4B). The two free juice populations followed a normal distribution, but they overlapped in the 20–32% range. This region of overlap included less than 10% of the juicy fruit population and less than 5% of the mealy fruit population.

The extractable juice content in the mealy 'O'Henry' fruit population ranged from 32 to 60%, with a mean of 45% and a standard deviation of 8.1% (Fig. 5A). The extractable juice content in the juicy 'O'Henry' fruit population ranged from 39 to 65% with a mean of 54% and a standard deviation of 6.7% (Fig. 5B). The two extractable juice populations followed a normal distribution, but they overlapped in the 39–59% range. This range included less than 99% of the juicy fruit population and less than 75% of the mealy fruit population.

In our peach storage test, different parameters indicated mealiness development during the 3 weeks cold storage. California 'O'Henry' peaches developed visual FM symptoms after 2 weeks storage at 5 °C (Fig. 6A). However, trained judges were able to detect 'off-flavors' or mealy

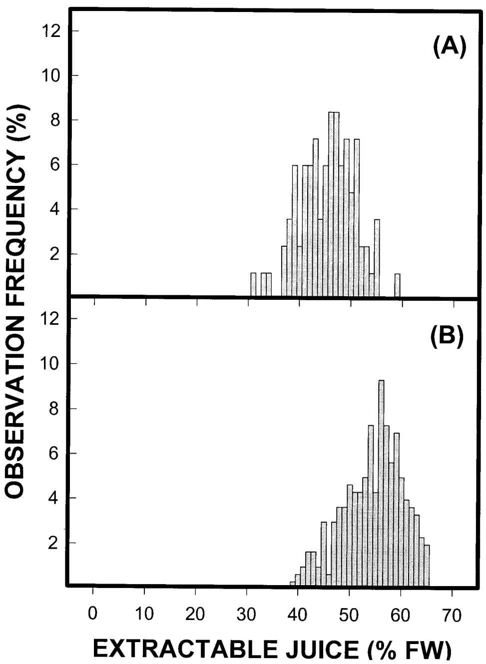

Fig. 5. Distribution of observed frequencies for 'O'Henry' peaches with different extractable juice percentages in the (A) mealy and (B) juicy categories according to our sensory trained panel.

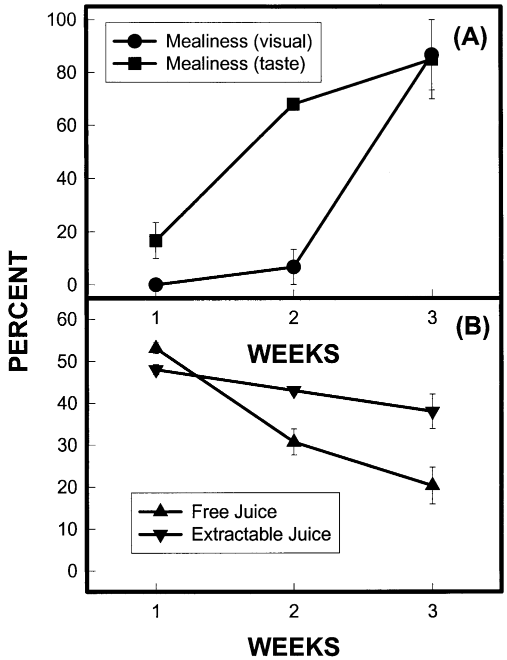

Fig. 6. 'O'Henry' peach flesh mealiness development during storage at 5 °C measured using different methods. Vertical bars represent ±SE.

texture 1 week before the visual mealiness symptom was apparent. Free juice content declined from 58% at harvest time to 28% after 2 weeks storage. According to our sensory evaluation (Fig. 6B), 'O'Henry' peaches with ≤32% free juice were almost always mealy. Thus, the percentage of free juice indicated that mealiness was likely to develop during ripening if fruits were subjected to 1–2 weeks of storage at 5 °C. On the other hand, percentage extractable juice did not change during the first 2 weeks of storage; it remained at approximately 46%, the same as at harvest. By the third week of storage, extractable juice decreased to approximately 35%. In all of the tests, our trained judges were able to perceive mealiness earlier than the visual symptoms were expressed. In some cases our trained judges were able to detect 'off flavor' even before the judges perceived mealy texture. This confirms that sensory evaluation should be added to a visual evaluation of mealiness to confirm chilling injury.

Our empirical comparison study demonstrates that a measurement of free juice content provides a quantitative, accurate and sensitive method to evaluate the onset of peach mealiness. The use of this quantitative method will likely make more meaningful comparisons of work done in different locations and years with fruit of a given cultivar. This method can be used for fundamental investigations of the onset of fruit mealiness, evaluation of breeding materials, and prediction of market life for different commercial cultivars.

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