Time to Fracture and Fracture Strain are Negatively Related in Sweet Cherry Fruit Skin

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ABSTRACT. Rain cracking of sweet cherry fruit (Prunus avium L.) is said to occur when the volume increase associated with water uptake, extends the fruit skin beyond its upper mechanical limits. Biaxial tensile tests recorded fracture strains (εfracture) in the range 0.17 to 0.22 mm² mm⁻² (equivalent to 17% to 22%). In these tests, an excised skin segment is pressurized from its inner side and the resulting two-dimensional strain is quantified. In contrast, the skins of fruit incubated in water in classical immersion assays are fractured at εfracture values in the range 0.003 to 0.01 mm² mm⁻² (equivalent to 0.3% to 1%)—these values are one to two orders of magnitude lower than those recorded in the biaxial tensile tests. The markedly lower time to fracture (tfracture) in the biaxial tensile test may account for this discrepancy. The objective of our study was to quantify the effect of tfracture on the mechanical properties of excised fruit skins. The tfracture was varied by changing the rate of increase in pressure (pₚ𝑟ₜᵃᶜᵗ𝑢ʳᵉ) and hence, the rate of strain (εₚᵣᵣᵦₑ) in biaxial tensile tests. A longer tfracture resulted in a lower pressure at fracture (pfracture) and a lower εfracture indicating weaker skins. However, a 5-fold difference in εfracture remained between the biaxial tensile test of excised fruit skin and an immersion assay with intact fruit. Also, the percentage of epidermal cells fracturing along their anticlinal cell walls differed. It was highest in the immersion assay (94.1% ± 0.6%) followed by the long tfracture (75.3% ± 4.7%) and the short tfracture (57.3% ± 5.5%) in the biaxial tensile test. This indicates that the effect of water uptake on cracking extends beyond a mere increase in fruit skin strain resulting from a fruit volume increase. Instead, the much lower εfracture in the immersion assay indicates a much weaker skin—some other unidentified factor(s) are at work.

Rain cracking limits the production of sweet cherries in all areas of the world when rainfall occurs during the harvest season (Christensen, 1996). Fruit cracking is thought to be related to water uptake into the fruit. When the volume of the fruit increases, the skin is strained, and this must eventually lead to mechanical failure. On the basis of this concept, water uptake into the fruit and the mechanical properties of the skin are the most critical factors in cracking.

Only a few studies have addressed the mechanical properties of the skin (Bargel et al., 2004; Brüggenwirth and Knoche 2016a, 2016b; Brüggenwirth et al., 2014). Because of the high Poisson’s ratio of the sweet cherry fruit skin, realistic experiments require biaxial tensile tests such as were first described by Bargel et al. (2004). In this test, an excised segment of the fruit surface is pressurized from its inner side. As a result, the segment bulges and its surface area increases thereby mimicking the increase in area associated with water uptake into the fruit (Brüggenwirth et al., 2014). Compared with the original protocol, the test procedure has been refined by maintaining the in vivo strain of the fruit skin and by pressurizing the exocarp segments (ES; synonym fruit skin segments) with silicone oil (AK10; Wacker Chemie, Munich, Germany) rather than with water (Brüggenwirth and Knoche, 2016a, 2016b; Brüggenwirth et al., 2014). Using this protocol, the pfracture were of similar magnitude to the turgor pressures reported for individual cells or those determined at the whole-fruit level (Knoche et al., 2014; Schumann et al., 2014). However, the εfracture (range 0.17 to 0.22 mm² mm⁻²) was consistently higher in the biaxial tensile tests than in immersion assays where fruit was immersed in water to determine its cracking susceptibility (Brüggenwirth and Knoche, 2016a). In these assays, a strain may be calculated from the water uptake required for 50% of the immersed fruit to crack. Typical (area) strain values in such immersion assays are in the order of 0.003 to 0.01 mm² mm⁻² (Brüggenwirth and Knoche, 2016a). The mechanistic basis for this difference in strain between the biaxial tensile tests and the immersions tests is unknown. One possible explanation would be the difference in the tfracture. In the immersions assays, fruit required on average at least 3 h to fracture, whereas in a biaxial tensile test, the ES fractured within ≈2 min. To our knowledge, the effect of tfracture on the mechanical properties of fruit skins is unknown.

The objective of this study was to quantify the effect of tfracture on the measured mechanical properties of excised sweet cherry fruit skins. We varied the tfracture by changing the prate in the biaxial tensile tests.

Materials and Methods

PLANT MATERIAL. Off-season ‘Sweetheart’ sweet cherry fruit from New Zealand were purchased locally and the fruit were selected for freedom from visual defects. The rootstock and cultivation system were unknown. Fruit was in excellent fully mature condition with turgent green pedicels. There were no signs of pitting or alligator skin or any symptoms of shriveling. Mature ‘Regina’ sweet cherry fruit was obtained from the horticultural research station of the Leibniz University at Ruthe...
Prunus cerasus rootstocks (Prunus cerasus L. × Prunus canescens Bois) and grown in a green house. Fruit were harvested at commercial maturity based on color and taste. Because the elastometer experiment was conducted during a 5-d period, fruit were placed in cold storage at 2 °C for up to 5 d. Fruit processed in a given experiment all originated from the same batch.

The elastometer. Biaxial tensile tests were carried out using an elastometer (Brüggenwirth et al., 2014). In this system, a skin segment is pressurized from its inner side causing the segment to bulge and to increase in surface area. A brass washer (12-mm inner diameter) was glued on one of the two shoulders of a sweet cherry fruit using a cyanoacrylate adhesive (Loctite 406; Henkel/Loctite Deutschland, Munich, Germany). An ES with a maximum thickness of 2.4 ± 0.02 mm was excised by cutting underneath the washer. Using this procedure, the in vivo strain of the skin was maintained until it was desired to increase it (Knoche and Peschel, 2006). The ES obtained in the washer comprised cuticle, epidermis, hypoderms, and adhering flesh cells (Brüggenwirth et al., 2014). The washer with the ES was mounted on the elastometer. The cuticle faced outward and the cut flesh surface was in contact with the silicone oil in the chamber. The ES was strained by pressurizing the silicone oil. This was achieved by driving a motorized piston into the chamber of the elastometer causing the ES in the washer to bulge outward. The pressure inside the chamber was recorded using a pressure transducer (type 40PC100G; Honeywell Intl., Morristown, NY) and the extent of bulging of the ES was recorded using a displacement transducer (KAP-S/5N; AST Angewandte System Technik, Wolnzach, Germany). The effect of $t_{\text{fracture}}$ was studied by varying the $p_{\text{rate}}$. The $p_{\text{rate}}$ was set at 0.001, 0.006, 0.04, 0.2, or 1 kPa s$^{-1}$. Following fracture, skin sections were prepared by excising tissue from one side of a crack using a razor blade. The sections were transferred to microscope slides and were viewed in incident light at ×250 (Axioplan; Carl Zeiss Microscopy, Jena, Germany). The fracture mode was quantified by counting the number of epidermal cells where fracture occurred along the anticlinal cell walls and expressing this number as a percentage of the total number of epidermal cells adjacent to the crack. For each treatment one crack per fruit on a total of 10 fruit per treatment were examined.

Data analysis. Occasionally, ES fractured at the edge of the orifice during biaxial tensile tests. Because of possible artifacts during mounting these ES were excluded from the analyses (Brüggenwirth et al., 2014). The data were subjected to analysis of variance. Percentage data were arcsine transformed before. Means were compared using Tukey’s studentized range test ($P<0.05$ (packet multicom 1.2–12, procedure glht, R 2.13.1; R Foundation for Statistical Computing, Vienna, Austria)]. Significance of the coefficient of determination ($r^2$) at the 0.05, 0.01, and 0.001 $P$ levels is indicated by *, **, and ***, respectively. Except for Fig. 1A where representative traces are shown, data are presented as means ± SE.

Results

Varying $p_{\text{rate}}$ in the elastometer yielded linear pressure/time relationships similar to the example in Fig. 1A, where in each, the slope of the line equals the $p_{\text{rate}}$. The arrows indicate the pressure and time of fracture. The $t_{\text{fracture}}$ was inversely related to $p_{\text{rate}}$ as indicated by the linear relationship of a log-log plot of time vs. rate [$\log$(time (h)) = $-0.92 (±0.014) \times \log$ rate (kPa s$^{-1}$) − 1.57 ± 0.025]; $r^2 = 0.90***$ (Fig. 1A, inset).

The stiffness of the ES as indexed by the $E$ increased as $t_{\text{fracture}}$ increased (Fig. 1B). In contrast, the $p_{\text{fracture}}$ and $e_{\text{fracture}}$ decreased at longer values of $t_{\text{fracture}}$ indicating that failures occurred at lower pressures and lower strains when tests were carried out over more extended periods of $t_{\text{fracture}}$ and, hence, over lower values of $p_{\text{rate}}$ (Fig. 1C and D). Relationships of mechanical properties with $t_{\text{fracture}}$ were log linear (Fig. 1B–D, insets). Hence, most changes in $E$, $p_{\text{fracture}}$, and $t_{\text{fracture}}$ occurred for values of $t_{\text{fracture}}$ less than 3 h, with little change in these parameters thereafter.

The time course of water uptake by submerged fruit was linear, at a constant rate of uptake of 29.7 mg h$^{-1}$ (Fig. 2, inset). For an average fruit of mass 13.1 g, assuming a spherical shape
and a density of 1 kg dm$^{-3}$, the cumulative water uptake of 44.6 ± 2.2 mg after 1.5 h corresponds to a strain of 0.002 mm$^2$ mm$^{-2}$. The percentage of cracked fruit increased in a sigmoidal pattern with time and with strain. The $t_{\text{fracture}}$ for 50% cracking was calculated at 5.17 h and the corresponding $\varepsilon_{\text{fracture}}$ was calculated at 0.008 mm$^2$ mm$^{-2}$ (Table 1). Compared with biaxial tensile tests carried out at a similar strain rate of the ES, the $t_{\text{fracture}}$ as measured by water uptake was only about one-third of that in the biaxial tensile tests, and the $\varepsilon_{\text{fracture}}$ was only about one-fifth of it (Table 1).

Comparing low (0.001 kPa s$^{-1}$) and high $p_{\text{rate}}$ (1 kPa s$^{-1}$) in the elastometer and data from the classical immersion assay in ‘Regina’ revealed that the mode of fracture differed between treatments. The percentage of epidermal cells that fractured along their anticlinal cell walls was highest in the immersion assay (94.1% ± 0.6%) followed by the low (75.3% ± 4.7%) and the high $p_{\text{rate}}$ (57.3% ± 5.5%). These differences were statistically significant.

**Discussion**

Our study produced two new and important findings: 1) A longer $t_{\text{fracture}}$ results in a higher value of $E$ (stiffer) but in lower values of $p_{\text{fracture}}$ and $\varepsilon_{\text{fracture}}$ (weaker) and a higher percentage of epidermal cells fracturing along rather than across their anticlinal cell walls and 2) the $\varepsilon_{\text{fracture}}$ in a biaxial tensile test carried out at comparable values of $\varepsilon_{\text{rate}}$ remains markedly higher than that estimated from an immersion assay.

**EFFECT OF TIME TO FRACTURE IN BIAXIAL TENSILE TESTS.** The mechanical properties of the ES were clearly affected by the $t_{\text{fracture}}$. Several explanations may be offered. First, most biological materials exhibit viscoelastic properties in their stress/strain relationships (Niklas, 1992). This includes fruit skins (Brüggenwirth et al., 2014; Hankinson et al., 1977) and fruit cuticles (Lopez-Casado et al., 2010; Matas et al., 2004; Petracek and Bukovac, 1995). In polymer science, viscoelasticity...
is described in analogical terms using rheological models comprising springs and dash pots arranged in series (Maxwell model), in parallel (Voigt–Kelvin model) or in combinations of both arrangements [Burgers model (Schmiedel, 1992)]. When subjected to a tensile force, stress/strain relationships are obtained that depend on the rate at which the force is applied. In sweet cherry fruit, epidermal and hypodermal cell layers, and not the cuticle, represent the load-bearing structure (Brüggenwirth et al., 2014). Furthermore, the plasma membrane restricts the movement of water as indicated by a decrease in skin stiffness after eliminating cell turgor (Brüggenwirth and Knoche, 2016b). Thus, mechanical properties of the fruit skin reflect those of the cell walls both from a material (composition) and a structural (cells with restricted water movement) point of view (Brüggenwirth and Knoche, 2016b). Therefore, applying the above analogy to cell walls of the sweet cherry skin, it may be speculated that the cellulose represents the spring, whereas the viscous pectins of the middle lamellae and the cellular structure (due to the restricted water movement) represent the dash pot (Vincent, 1990). Consequently, at a high ε_rate we would expect the cellulose to be stressed and strained, whereas at low ε_rate the middle lamella is strained and water movement from symplast into the apoplast is induced. This hypothesis would explain why varying t_fracture resulted in different stiffness, p fracture and ε fracture. In addition, this explanation would also account for the observed differences in failure mode where a higher percentage of epidermal cells failed along their anticlinal cell walls at the lower p_rate. That the mode of failure of tissue may be affected by other factors such as turgor is not unusual as De Belie et al. (2000) demonstrated for mechanical properties of pear parenchyma.

Second, formation of cracks may depend on time. For a short t fracture a crack may initiate rapidly at low pressure but crack extension and complete failure may require more time and thus, occurs later when the pressure is already higher. This argument would account for the effect of t fracture in the biaxial tests; i.e., the linear relationship on the log scale between mechanical properties and t fracture. Also, the difference between the biaxial tensile test and the immersion assay may be accounted for by the above argument.

TENSILE TEST VS. IMMERSION ASSAY. The calculated ε fracture and t fracture (Table 1) in the immersion assay are of the same order of magnitude as those of earlier studies (Christensen, 1996; Winkler et al., 2015). However, comparison of biaxial tensile tests for long t fracture with the immersion assay, reveals that the ε fracture still remains higher in the tensile test. This effect was not unique for off-season ‘Sweetheart’, but could be reproduced with freshly harvested ‘Regina’. The difference in

Table 1. Comparison of mechanical properties of ‘Sweetheart’ sweet cherry fruit skin established in biaxial tensile tests at two different rates of increase in pressure and hence of strain vs. immersion assays of whole fruit. Mechanical properties were indexed by the time to fracture (t fracture) and the strain at fracture (ε fracture). For the immersion assay, strain rates (ε rate) were calculated from water uptake rates into the fruit. The ε rate in biaxial tensile tests was measured from the increase in surface area associated with the rate of increase in pressure (p rate).

| Treatment          | p_rate (kPa·s⁻¹) | ε_rate (mm²·mm⁻²·h⁻¹) | t fracture (h) | ε fracture (mm²·mm⁻²) |
|--------------------|-----------------|-----------------------|---------------|---------------------|
| Biaxial tensile test | 1               | 4.0875 ± 0.0000 a'     | 0.027 ± 0.002 c' | 0.199 ± 0.008 a'    |
| Biaxial tensile test | 0.001           | 0.0025 ± 0.0006 b     | 16.68 ± 0.743 a | 0.041 ± 0.004 b     |
| Immersion assay    | –               | 0.0015 ± 0.0004 b     | 5.17 ± 0.385 b  | 0.008 ± 0.001 c     |

Mean separation by Tukey’s studentized range test at P < 0.05.

ε fracture may be explained by one or several of the following factors. First, the effect of water uptake somehow extends beyond its effect on the strain of the skin that is caused by the volume increase of the fruit. For this to happen, the water taken up directly or indirectly must somehow affect the cell walls causing the mode of fracture to change. For example, an indirect effect could be the bursting of individual epidermal cells that would result in leakage of malic acid into the cell-wall space. Malic acid, in turn, has been shown to increase the permeability of the plasma membrane and to further weaken cell walls (Winkler et al., 2015). This could result in the spreading of a defect similar to a “zipper” or to a “ladder” in a piece of knitted fabric. Second, spatial heterogeneity of water uptake may lead to localized strains that exceed the average strain calculated on the basis of whole-fruit water uptake. This could result in local ε fracture values, comparable to those of the biaxial tensile tests, whereas the mean values calculated on a whole-fruit basis would be much lower. However, there is no indication that preferential sites of water uptake are involved. In the immersion assays, water uptake was restricted to the fruit surface by sealing the pedicel junction with silicone rubber (Beyer et al., 2002). Third, fruit in the immersion assay usually cracked in the stylar scar region, whereas the ES in the biaxial tensile test was excised from the shoulders of the fruit. However, there was no difference in the mechanical properties between the shoulder and the stylar scar in earlier studies making the above argument less likely (Brüggenwirth and Knoche, 2016b). Fourth, an artifact caused by silicone oil permeating the tissue and weakening the cell wall in the elastometer is unlikely. When establishing the biaxial tensile test, we pressurized cell walls using water and silicone oil and found no significant difference between the two at the standard p_rate. However, to avoid artefacts due to cell bursting in water during longer exposure times, silicone oil was used as a standard. Finally, it may be visualized that water uptake into epidermal cells increased cell turgor in fruit in the immersion assay. To our knowledge, there is no supporting evidence for this hypothesis in the literature. Cell pressure probe (CPP) techniques are too coarse to puncture the small epidermal cells and obtain direct measurements of turgor. In our earlier study, we found no effect of water uptake on cell turgor of mesocarp cells as determined by CPP (Knoche et al., 2014). At present, the mechanistic basis for the difference in mechanical properties is not known.

Conclusions

The data demonstrate that t fracture affects the mechanical properties and the mode of fracture recorded for the ES. Part of the difference in ε fracture between the biaxial tensile tests and the immersions assays is accounted for by the difference in t fracture. However, some discrepancies remain, indicating that the volume increase caused by water uptake is not the only factor involved. Apparently, water uptake affects the ES in other ways, which lead to failure at lower ε fracture than predicted based on biaxial tensile tests. In addition, a higher percentage of fracture of epidermal cells along their anticlinal cell walls is
observed in the immersion assay. The mechanistic basis of both effects is unknown. Because the mechanical properties of the ES are determined primarily by the cell walls (Brüggenwirth and Knoche, 2016b), further studies should focus on the interaction between water uptake and the mechanical behavior of the cell wall.

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