Tomato (*Lycopersicon esculentum* Mill.) fruit growth and ripening as related to the biomechanical properties of fruit skin and isolated cuticle

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**Abstract**

The control of growth rate and the mechanical integrity of the tomato (*Lycopersicon esculentum* Mill.) fruit has been attributed to the exocarp. This study focused on the biomechanics of the fruit skin (FS) comprising cuticle, epidermis and a few subdermal cell layers, and the enzymatically isolated cuticular membrane (CM) during fruit growth and ripening. Morphology and mechanical properties of the FS and the CM of three cultivars were analysed separately at three distinct ripening stages by scanning electron microscopy (SEM) and one-dimensional tension testing, respectively. Both were subject to significant cultivar-specific changes. Thickness of the CM increased during ripening from 7.8–8.6 to 9.9–15.7 μm and exceeded by far that of the epidermal cell wall. The mechanical properties, such as modulus of elasticity, strength, and failure strain, were highest in the FS for all cultivars at any stage, with only one exception; however, the cuticle largely mirrored these properties throughout fruit maturation. Stiffness of both isolated CM and FS increased from immature to fully ripe fruits for all cultivars, while failure stress and failure strain displayed a tendency to decrease for two of them. Stress–strain behaviour of the CM could be described as strain softening, mostly linear elastic throughout, strain hardening, and was subject to growth-related changes. The FS displayed strain hardening throughout. The results indicate evidence for the cuticle to become increasingly important as a structural component for the integrity of the tomato fruit in addition to the epidermis. A supplementary putative model for tomato fruit growth is proposed.

**Key words:** Cuticle, cracking, epidermis, fruit growth, *Lycopersicon esculentum*, plant biomechanics, ripening, stiffening, tomato.

**Introduction**

Fruit growth and ripening are complex developmental processes that involve multiple metabolic changes. The climacteric berry fruit type of tomato (*Lycopersicon esculentum* Mill.) is the most intensively studied model species (Giovannoni, 2001), thus representing a good system for biomechanical studies as related to growth and ripening. The mechanical performance of the exocarp, or fruit skin, including the cuticle, the epidermis, and a variable number of hypodermal cell layers, is of considerable economic significance for the integrity of the whole fruit. It affects not only fruit appearance, handling, and storage (Chu and Thompson, 1972), but also plays a prominent role in fruit cracking (Sekse, 1995; Emmons and Scott, 1997; Bertin et al., 2000). Moreover, it is generally accepted that the growth rate of plant tissues is regulated by the interaction of cell wall stress and cell wall mechanical properties (Cosgrove, 1993), and it has been argued that tissue tension in tomato fruits indicates that the fruit epidermis is of importance in determining the rate of expansion (Thompson et al., 1998). During the ripening process, cell wall-modifying activity of several enzymes, including polygalacturonase, pectin-methyl-esterase, endo-β-mannase, α- and β-galactosidases, and β-glucanases, causes softening of the whole fruit by altering the texture due to degradation of the structural components necessary to reinforce the cell wall and the adhesion of cells (Fischer and Bennett, 1991; Rose...
et al., 1997; Seymour et al., 2002). Expansins have been described as contributing significantly to ripening-related fruit softening (McQueen-Mason, 1995), and may affect both cell wall viscosity and elasticity of tomato fruit epidermis (Thompson, 2001). On the other hand, peroxidase-mediated stiffening of the tomato fruit skin cell walls has been proposed as a control mechanism for fruit growth (Thompson, 2001; Andrews et al., 2002a, b), and may counteract the softening of the inner fruit tissues. Thus, knowledge of the mechanical properties of the outer fruit envelope is of vital interest, and several studies have been carried out, mostly by measuring the mechanical properties of the tomato fruit skin without distinguishing its components (Batal et al., 1970; Voisey et al., 1970; Thompson, 2001; Andrews et al., 2002b).

By contrast, there is only a limited number of studies that have aimed at characterizing the mechanical properties and behaviour of the isolated tomato fruit cuticle (Petracek and Bukovac, 1995; Wiedemann and Neinhuis, 1998; Bargel et al., 2000). The plant cuticle is a natural composite that covers the epidermis as a continuous extracellular membrane, consisting mainly of two components, the insoluble biopolymer cutin and soluble lipids, collectively called ‘waxes’ (Kolattukudy, 1980). Minor amounts of cell wall polysaccharides are gradually encrusted during development and link the cuticle to the epidermis (Holloway, 1994). A second, highly resistant biopolymer named cutan originally found in fossilized cuticles has not been detected in tomato fruit cuticle (Ramírez et al., 1992). The polymeric plant coverage appears to be without uniform ultrastructure and chemical composition, and differences across and within species have been demonstrated (overviews in Jeffree, 1996; Kolattukudy, 2001). Moreover, Baker et al. (1982) have studied the chemical composition of the cuticle in relation to tomato fruit development and ripening, and have reported distinct dynamic changes of both cutin and cuticular wax composition. The same was proposed for phenolic compounds (Hunt and Baker, 1980). The plant cuticle is a multifunctional interface that primarily prevents the plant from uncontrollable water loss (Schönherr, 1982; Riederer and Schreiber, 2001), but also serves as a protective layer against multiple biotic and abiotic environmental influences (Bargel et al., 2004a). From a mechanical point of view, the location at the outer perimeter of the tomato fruit indicates that the cuticle may function as an external structural element that adds mechanical support for tissue integrity, since engineering theory shows that stresses are highest at the surface of a body (Niklas, 1992).

In this context, Wiedemann and Neinhuis (1998) studied the mechanical properties of isolated cuticles from five leaf species and mature tomato fruit by means of one-dimensional tension. Recently, Matas et al. (2004) analysed the mechanical properties of fruit peels and isolated cuticle of ripe tomato fruits, and stated that the cuticle is a mechanically important component of the tomato fruit. As far as is known, integrative studies of both the cuticle and underlying cell wall of a plant organ related to growth and ripening are not available.

This paper focuses on the tensile properties of both enzymatically isolated cuticular membrane (CM) and peeled fruit skin (FS) of tomato fruits as related to fruit growth and ripening, i.e. at three distinct ripening stages. Three cultivars were used which differed in cracking susceptibility and fruit shape. The objectives were to evaluate (i) changes of the mechanical properties of both CM and FS, and (ii) the contribution of the CM to the mechanics of the outer fruit envelope and, hence, the integrity of the whole organ during fruit growth and ripening. Concurrent scanning electron microscopy was carried out for morphological characterization.

Materials and methods

Plant material

Three tomato cultivars, cv. Harzfeuer (eight plants), cv. Vanessa F1 (six plants; both oblate-spherical) and cv. Roma (five plants; oblong), were grown in parallel rows on a 1×5 m patch from commercially available seeds under greenhouse-conditions without supplemental lighting during the summer period (Experimental Station, Botanical Gardens, University of Bonn). Cultivar-specific differences were expected by comparing three cultivars with different genetic backgrounds, while cv. Harzfeuer was chosen as a cracking-susceptible cultivar. Plants were irrigated manually every second day, and fertilization followed a regular scheme every second week throughout the season with a standard mineral nutrition solution. Plants were reduced to six to eight trusses, and trusses were pruned to a maximum of six fruits. Fruits with diameter >30 mm were harvested at three distinct ripening stages, i.e. immature-green (ig), mature-green (mg), and mature-red (mr), following the nomenclature of Emmons and Scott (1997). The first fruits that appeared per truss were left on the plant until full maturity, and subsequent fruits were then harvested at the appropriate stage during the course of 4 weeks. The classification of the ripening stages was made on the relative parameters of size, weight, and colour change after the ‘breaker’ stage, rather than by days from anthesis. This classification may introduce inaccuracies due to cross-cultivar variation of fruit maturation, however, with the cultivars used, the classification system sufficiently matched uniform ripening stages (Table 1). Fruit size dimensions were obtained using a gauge, and fruit weight was measured individually with a digital laboratory balance at 0.1 g resolution. A representative assembly of the three cultivars at the corresponding ripening stages is shown in Fig. 1.

Parallel cut samples (sample size: 3×40 mm) were obtained with a custom-built double-blade device, with cutting direction from the peduncle insertion to the tip. These peel samples were divided into two batches of similar size. Cuticular membranes (CM) were then isolated enzymatically using a modified cellulase/pectinase solution after Wiedemann and Neinhuis (1998), containing cellulase (1.75% [w/w], Cellulac; Novo Nordisk, Bagsvaerd, Denmark), pectinase (3.5% [w/w], Fluka, Buchs, Swiss), sodium azide (NaN3, 2 mM; Merck Schuchardt, Hohenheim, Germany), polyvinyl pyrrolidone (0.2% [w/w]) and citric acid monohydrate (20 mM; Merck, Darmstadt, Germany) at 30 °C. A pH of 5.0 was established for optimum enzyme activity. Completely isolated CM were rinsed twice in distilled water, air-dried on Teflon meshes, and stored in Petri dishes at room temperature before testing. Corresponding fruit skin (FS) samples were prepared from the peeled strips by carefully removing adhering
parenchyma tissue with a razor blade. To eliminate physiological stress reactions and an effect of turgor during mechanical testing, FS were incubated for 1 min in ethanol (80%; Roth, Hamburg, Germany), and subsequently washed for 5 min in distilled water, using polyvinyl pyrrolidone/Na$_2$O$_3$ (0.2% [w/w]/2 mM) as storage medium until testing. The frequently used freeze–thaw technique in extensibility studies was excluded since earlier preliminary studies on tomato and cherry (Prunus avium L.) fruit indicated that freezing could produce micro-cracks in the cuticle. In addition to the above, a preliminary study including fruit material of cv. Vanessa and cv. Roma was conducted in an earlier growth season. The CM was isolated similarly, but the FS was tested fresh without eliminating physiological stress reactions prior to mechanical characterization.

**Scanning electron microscopy**

Following the conclusions of Hill and Dilcher (1990), scanning electron microscopy (SEM) combined with digital image analysis of cross-sections was used for morphological characterization and determination of sample thickness, from which the corresponding cross-sectional area (thickness×width of the sample) necessary for the calculation of stress was derived. FS samples were fixed with methanol (99.9%; Fluka, Darmstadt, Germany) according to Neinhuis and Edelmann (1996) for 1 min, and subsequently critical-point-dried (CPD 020; Balzers Union, Wiesbaden, Germany), and subsequently examined on a modified aluminium stub at 15 kV (440i; LEO, Oberkochen, Germany). Digital image analysis was carried out with the free NIH Image software (http://rsb.info.nih.gov/nih-image/). For the FS, thicknesses of the cuticle and underlying epidermal cell walls were determined by scanning a selected range of each cross-section, excluding cell lumen as well as the hypodermal cell layers. Wherever possible, the inner epidermal part of the double wall was differentiated from the part belonging to the subepidermal cells. In general, the distinction between the cuticle and the epidermal cell wall was easily distinguished in the preliminary studies using SEM analysis. Thickness measurements of the CM covered all cutinized layers of the enzymatically isolated cuticular material. Measurements were carried out with at least eight replicates of both CM and FS per fruit (n=3–5) and ripening stage of each cultivar, and the results are given as means ± confidence intervals (CI).

**Mechanical tests**

Mechanical characterization of fully hydrated samples was carried out by means of one-dimensional tension testing with a custom-built computer-controlled device featuring vice-type clamps, as has been described by Wiedemann and Neinhuis (1998). Accordingly, the test speed was set to 2 mm min$^{-1}$. CM samples were rehydrated, and both CM and FS samples were kept moist during testing by adding small amounts of distilled water with a hand pipette to mimic a fully hydrated water status of both cell walls and cuticle. Effectiveness of rehydration of the CM was not quantified, but was considered to ensure fully hydrated CM in only a few minutes after adding water. This has been shown in the authors’ laboratory for a large variety of CM from different species, while the mechanical properties changed immediately (H Bargel, C Neinhuis, unpublished results). From the tensile tests, the modulus of elasticity $E$ (stress/strain), conventional breaking stress $\sigma$ (force/cross-sectional area), and conventional breaking strain $\epsilon_{br}$ (change of length/initial length) were derived. Most of the stress–strain curves displayed an initial and a second linear slope, resulting in two different moduli of elasticity, as well as in a transition point between the first and the second slope, which was equivalent to the proportional limit for part of the CM measurements. In addition, modulus, stress, and strain values were also calculated as true stress $\sigma_t$ and strain $\epsilon_t$, since plant polymers can change their form to a great extend (>10%) over a relatively short period of time (Vincent, 1990b). Hence, calculation of conventional stress and strain could produce underestimated and overestimated values, respectively. This may be illustrated as follows: if the extension of any material (with a known initial length $l_0$) above 10% total length increase is considered, the same incremental increase in length will produce a smaller true strain than the conventional equivalent, since the true strain provides a reference strain for each increment of extension relative to the preceding total deformation. By contrast, the conventional strain is always referred to $l_0$, irrespective of the total deformation. In a similar way, the cross-sectional area used for the calculation of stress defines the differences between true stress and conventional stress. Calculations of true stress and strain were related to the conventional numbers for the starting conditions as has been proposed by Biewener (1992), assuming isovolumetric deformation (Shadwick, 1992).

<table>
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<th>Horizontal ø</th>
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<td>4.6</td>
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**Fig. 1.** Compilation of representative tomato fruits of the cvs Vanessa F1 (top), Harzeufer (middle), and Roma (bottom) at three distinct ripening stages. From left to right: immature-green, mature-green, and mature-red.
\[ \sigma_i = (1 + c)\sigma \]  
\[ \varepsilon_i = \ln(l/l_0) \]

Measurements were carried out with at least eight replicates of both CM and FS per fruit \((n=3-5)\) and ripening stage of each cultivar. In the Results section, means and confidence intervals (CI) are given for modulus of elasticity and transition point. For strength and breaking strain, trend values are given instead, since a large number of samples failed close to the clamps, which made reproducible results difficult (Wiedemann and Neinhuis, 1998). This phenomenon is commonly observed in tensile tests and is caused by shear stresses perpendicular to the tensile stresses near the clamping site. While stiffness is not affected, fracturing close to the clamps may occur at lower stress and strain at failure. In this study, these measurements were included, and trend values reported here do account for this problem.

**Statistical analysis**

An unbalanced two-way ANOVA with Bonferroni post-test (Prism 4; GraphPad, San Diego, California, USA) at \(\alpha = 0.05\) was performed on thickness and modulus of elasticity in order to evaluate significant changes of either morphology or stiffness related to fruit ripening. An outlier test after Nalimov (Lozán and Kausch, 1998), as well as a normality test, were carried out prior to the statistical analysis.

**Results**

**Morphology**

SEM analysis of both CM and FS cross-sections revealed overall thicker cuticles for all cultivars compared with epidermal cell walls at any stage of ripening. Representative cross-sections of the CM and FS at all three ripening stages are shown in Fig. 2. No adhering cellular material is visible on the inner fasciae of the CM, indicating complete enzymatic isolation of the extracellular biopolyester membrane. Differentiation between the cuticular material and the cell walls generally could be clearly depicted. The shape of epidermal cells changed from more or less isodiametric towards elongated during development. This was manifested not only in the FS, but also in the isolated CM. The fracture surfaces of both CM and FS are of mostly uniform texture, with slightly smoother surfaces at the earlier stages of ripening for all cultivars. Cuticular encasing of epidermal cells was typically absent in stage ig, but increasingly abundant during ripening regardless of cultivar, resulting in an overall thicker CM at ripening stages mg and mr. This was particularly the case for cv. Vanessa. Gain in mean thickness of CM from immature to fully mature fruits was highly significant \((P < 0.001)\) for all cultivars, with considerable differences among the cultivars (Fig. 3). In detail, cv. Harzfeuer increased from 8.7 ± 0.3 \(\mu\)m to 10.2 ± 0.6 \(\mu\)m, cv. Vanessa almost doubled from 8.7 ± 0.5 \(\mu\)m to 15.7 ± 0.6 \(\mu\)m, and cv. Roma enlarged from 8.4 ± 0.4 \(\mu\)m to 9.6 ± 0.7 \(\mu\)m. However, thickness of CM of cv. Harzfeuer was highest at stage mg and then dropped towards stage mr. Further analysis of the CM in order to evaluate the contribution of the outer solid part of the cuticle to overall CM thickness revealed a highly significant increase \((P < 0.001)\) of thickness from 4.6 ± 0.1 \(\mu\)m at stage ig to 5.4 ± 0.1 \(\mu\)m at stage mr for cv. Harzfeuer, and from 4.9 ± 0.1 \(\mu\)m to 8.1 ± 0.3 \(\mu\)m for cv. Vanessa F1. A non-significant increase from 5.3 ± 0.1 \(\mu\)m to 5.5 ± 0.1 \(\mu\)m was measured for cv. Roma (Fig. 4).

By contrast, thickness of the epidermal cell walls showed no uniform trend. While cv. Roma showed a very significant gain \((P < 0.01)\) from 1.0 ± 0.04 \(\mu\)m at stage ig to 1.1 ± 0.07 \(\mu\)m at stage mr, cell wall thickness of cv. Vanessa dropped highly significantly \((P < 0.001)\) from 1.3 ± 0.07 \(\mu\)m to 1.1 ± 0.04 \(\mu\)m, and cv. Harzfeuer remained constant at 1.3 \(\mu\)m (Fig. 3).

**Biomechanical properties**

Tensile measurements demonstrated considerable changes in the mechanical properties of the outer fruit envelope during fruit maturation in a cultivar-specific manner, as well as differences between the CM and FS (Fig. 5). A stress–strain analysis revealed three forms of stress–strain behaviour for the tomato fruit CM with persistent patterns worthy of further analysis. Representative examples are given in Fig. 5A. The most obvious tendency was strain hardening material behaviour (increasing modulus) at stage mg for all cultivars, whereas stage ig could be described as a mixture of strain softening (decreasing modulus) and a mostly linear form. At maturity, stress-strain behaviour was not that uniform, and cultivar-specific differences could be noted. Here, cv. Harzfeuer showed a mixture of strain softening and mostly linear form, while a mostly linear form and strain hardening for a large part of the stress–strain curves could be depicted for the majority of CM samples of cv. Vanessa. For cv. Roma, a mostly linear form dominated. By contrast, the stress–strain behaviour of the FS could be described as strain hardening up to approximately 6–9% strain throughout all ripening stages (Fig. 5B), the exception being cv. Roma stage mr which displayed linear behaviour. Two linear slopes were identified in all diagrams showing strain softening and strain hardening and, consequently, transition points between primary and secondary slopes could be defined (Köhler and Spatz, 2002).

From the individual stress–strain diagrams of each measurement, the mechanical properties of the CM and the FS were calculated. The modulus of elasticity, a measure for tensile resistance or stiffness, was found to be highest for the FS of all cultivars throughout ripening, with the exception of stage mr of cv. Harzfeuer (Fig. 6). However, modulus changes of the FS as related to ripening had no uniform trend. Stiffness of the FS of cv. Roma increased highly significant \((P < 0.001)\) from 141.6 ± 17.7 MPa at stage ig to 292.6 ± 36.0 MPa at stage mr, while that of cv. Harzfeuer displayed a non-significant increase from...
204.4 ± 23.4 MPa to 251.8 ± 47.6 MPa. The FS of cv. Vanessa F1 showed a non-significant decrease in stiffness from 296.7 ± 59.2 MPa to 258.3 ± 12.0 MPa (Fig. 6). In contrast to the FS, the CM of all three cultivars displayed a significant species-specific increase of the modulus of elasticity from stage *ig* to *mr*, although generally at lower values. The only exception was CM of cv. Harzfeuer at full maturity, which superseded the modulus of the corresponding FS (Fig. 6). This ripening-related increase was highly significant (*P* < 0.001) for both cv. Harzfeuer from 117.0 ± 23.9 MPa to 258.0 ± 66.1 MPa, and for cv. Roma from 96.7 ± 7.7 MPa to 191.8 ± 50.7 MPa. A significant increase (*P* < 0.05) could be determined for cv. Vanessa F1 from 175.2 ± 39.8 MPa to 209.3 ± 17.3 MPa, respectively.
A small decrease in CM stiffness of immature to mature-green fruits was common for all cultivars. As far as stress and strain at failure are concerned, both the CM and the FS displayed a trend to have the lowest values at full maturity in all cultivars, with the notable exception of FS of cv. Roma. Conversely, the CM of mature-green fruits of all cultivars displayed the highest strength as well as strain, with the sole exception of cv. Vanessa F1, where the highest strain was found at stage mg (Table 2). Comparison of the conventional values with the true analogues revealed moderate differences (Table 3). The modulus of elasticity of both CM and FS was 10–15 MPa higher when calculated as true stress versus strain. True stress at failure of the CM exceeded that of the conventional values up to 2 MPa, and that of the FS about 2–4 MPa, while true failure strain of the CM was up to 1% lower, and that of the FS about 1–3%, respectively.

**Discussion**

*Morphological changes related to fruit ripening*

Morphology of the fruit exocarp, in particular of the cuticle, of all three cultivars was subject to significant changes during fruit maturation, resulting in extensive cutinization of epidermal cell walls at full maturity. The change in cellular shape indicated that higher tensile stresses were exerted on the exocarp at the later stages of maturity, according to the stress distribution during fruit growth (Considine and Brown, 1981; Thompson et al., 1998). CM thickness at maturity of the three cultivars studied here are consistent with other reports, for example, 6.7–15.3 \( \mu m \) for ripe tomato fruit (Petracek and Bukovac, 1995). In this latter study, thickness of the solid outer CM region ranged between 4.4–10.7 \( \mu m \), which is fairly consistent with the range of 4.6–8.1 \( \mu m \) determined here.

An increase in thickness has to be compensated by biosynthesis of material throughout growth and ripening, as has been demonstrated for tomato fruit cuticle by Baker et al. (1982). The gain in thickness of the CM as related to fruit growth could mainly be assigned to the accumulation of cuticular compounds at the outer solid part, as well as
Concerning the changes in cell wall thickness of the FS, the results were within the range of 0.66–4.40 μm reported for five different tomato cultivars by Borowiak and Habdas (1988), but indicated a certain degree of variability among the cultivars tested. The same mechanisms as stated for the CM are very likely to determine thickness changes of the cell wall of the FS. On the one hand, a growth-related increase in thickness as shown for cv. Roma might reflect a more or less continuous biosynthesis of new cell wall material. By contrast, thinner cell walls at stage mr, as was the case with cv. Harzfuer and cv. Vanessa, might be due to growth-related stress during rapid expansion, while accumulation of wall material is persistent at different activities until the final stages of ripening, where growth generally ceases (Monseline et al., 1978; Thompson et al., 1998).

Mechanical properties related to fruit ripening

The mechanical properties of the CM and FS were also subject to changes for all cultivars during fruit growth and ripening. The modulus of elasticity of both the CM and the FS generally increased during ripening, while strength and strain at failure showed a tendency to decrease. The reported moduli of all cultivars were in the upper range compared with other studies. Wiedemann and Neinhuis (1998) reported a modulus of elasticity between 60–100 MPa for the CM of fully ripe tomato fruits, depending on the hydration status, and Matas et al. (2004) noted stiffness values of ripe tomato fruit CM of cv. Inbred 10 and cv. Sweet 100 of 70 MPa and 53 MPa. A similar growth-related increase in tomato fruit skin tensile resistance as observed in this study was recently reported with values ranging from about 30–110 MPa (Andrews et al., 2002b). Other available studies on fruit skins reported modulus values between 10–20 MPa for different tomato cultivars (Batal et al., 1970), and 600 MPa for tomato cv. Beefsteak (Vincent, 1990a). Concerning strength and strain at failure, values for skins between 0.9–1.7 MPa and 7.8–13.9% (Batal et al., 1970) as well as 9.6–15.5 MPa and 28.5–29.2% (Voisey et al., 1970) for different tomato cultivars, and 2.5 MPa and 23.5% for cv. Beefsteak (Vincent, 1990a) can be found in the literature. Andrews et al. (2002a) noted stress and strain values at maximum load during the course of fruit growth of cv. Espero to increase from about 5 MPa to 10 MPa and to decrease from about 24% to 10%, respectively. However, Matas et al. (2004) reported comparably low breaking stress values between about 1–1.2 MPa for the CM of ripe tomato fruits. The compliance of isolated CM from mature tomato cv. Pik Red in a load-creep test has been analysed, with a strain at failure of about 3% (Petracek and Bukovac, 1995), which is in very good agreement with strain of 3–5% at full maturity for the three tested cultivars in this study. A possible reason for the rather large discrepancies between the modulus and strength values of the FS in this study and the literature might be the determination of sample thickness. If the cell lumens of the cross-sectional areas were included, i.e. by measuring thickness crudely with a pair of callipers, lower stress and stiffness values would be the outcome. In this study, cross-sectional area of the FS was based on the thickness of the epidermal cell walls excluding the hypodermal cells, since the epidermis is likely to be mechanically more important for tensile resistance, breaking stress and breaking strain. There are studies available that give some support for this assumption (Thompson,
2001; Matas et al., 2004). However, the hypodermal cell layer(s) may support the skin on the fruit, for example, by energy absorption (Bargel et al., 2004b; Matas et al., 2004). The methanol fixation technique used in conjunction with critical-point-drying has been shown to produce very few shrinking artefacts of tissues and cell wall components (Neinhuis and Edelmann, 1996), making it a favourable method for SEM and image analysis of the fruit exocarp components, as has also been successfully demonstrated for cherry fruit by Bargel et al. (2004b).

As far as true stress and strain values are concerned, only very few data for biological materials exist so far (Niklas, 1992). As far as is known, no study on tomato fruit skins is available, making comparison with other data somewhat difficult. The differences between stiffness calculated from conventional as well as true stress and strain were obviously small in the range exhibited by the cultivars studied, and only a little higher for stress and strain at failure. Although it may be a counsel of perfection, true stress and strain could provide a more accurate picture of what is happening in a material, particularly far above 10% strain (Vincent, 1990b). It would be even more favourable to calculate these numbers without relating them to the starting conditions, as was done in this study.

**Table 2.** Strength as normal breaking stress (MPa), strain as normal breaking strain (%), and transition point (TP; %) of the cuticular membrane (CM) and the fruit skin (FS) of three tomato cultivars as related to fruit growth and ripening

TP represents the transition point from the first into the second slope of the stress–strain curves. Values for strength and strain represent trends, while means ±95% CI are given for the TP: ig, immature-green; mg, mature-green; mr, mature-red.

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</table>

**Table 3.** Modulus of elasticity (MPa), strength (MPa), and strain (%) of the cuticular membrane (CM) and the fruit skin (FS) of three tomato cultivars as related to fruit growth and ripening

Calculations are based on true numbers after equations (1) and (2). Although the absolute values differed, changes in the mechanical properties as related to fruit maturation remained stable. Means ±95% CI are given for the modulus, while values for strength and strain represent trends: ig, immature-green; mg, mature-green; mr, mature-red.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Stage</th>
<th>Modulus (MPa)</th>
<th>Strength (MPa)</th>
<th>Strain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CM</td>
<td>FS</td>
<td>CM</td>
</tr>
<tr>
<td>Harzeuer</td>
<td>ig</td>
<td>131.6±9.1</td>
<td>≤12</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>mg</td>
<td>120.6±10.4</td>
<td>≤13</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>mr</td>
<td>267.2±67.9</td>
<td>≤3</td>
<td>4.4±0.7</td>
</tr>
<tr>
<td>Vanessa F1</td>
<td>ig</td>
<td>186.6±24.2</td>
<td>≤18</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>mg</td>
<td>141.7±7.7</td>
<td>≤16</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>mr</td>
<td>224.6±20.7</td>
<td>≤12</td>
<td>5</td>
</tr>
<tr>
<td>Roma</td>
<td>ig</td>
<td>100.7±8.0</td>
<td>≤5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>mg</td>
<td>99.5±19.1</td>
<td>≤16</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>mr</td>
<td>199.5±52.1</td>
<td>≤5</td>
<td>3</td>
</tr>
</tbody>
</table>

**Ripening-related changes of the mechanical properties of the FS**

Not surprisingly, the FS displayed a higher tensile stiffness as well as strength, but overall it also showed higher extensibility than the CM. Clearly, the epidermal cell walls contribute to a large extent to the mechanical properties of the tomato fruit exocarp, where the cellulose network is the load-bearing component (Vincent, 1990b; Niklas, 1992). It has been proposed that the epidermis plays a major role in resistance to turgor-driven tomato fruit growth (Ho, 1992; Thompson, 2001; Andrews et al., 2002b), since it is altered during the ripening process by cell wall-specific enzymatic activity. For example, the interplay between xyloglucan-endo-transglycosylase (XET) and peroxidase, their pattern of appearance, and their weakening and stiffening effects on the epidermal cell wall, respectively, have been described (Thompson et al., 1998; Andrews et al., 2000). Moreover, peroxidase-mediated stiffening of exocarp cell
walls have been described (Andrews et al., 2002a, b). This has also been proposed for maize (Zea mays L.) coleoptiles after hydrogen peroxide treatment (Schopfer, 1996). These ripening-related physiological modifications appear largely to account for the observed changes during fruit maturity in fruit skin tensile stiffness, but also to some extent of strength and strain at failure. However, the changes of the mechanical properties are not uniform for the tested cultivars, and thus cannot be categorically generalized. Whereas the results of cv. Roma agree well with the proposed model of epidermal cell wall strengthening, stress at failure values of cv. Harzfeuer and cv. Roma decreased during maturity. It is very likely that the peroxidase content and hence the stiffening effect varies in a cultivar-specific way. In addition, the ripening-induced activity of expansins and other enzymes such as endo-β-mannanase and poygalacturonase suggested to be involved in fruit softening (McQueen-Mason, 1995; Bewley et al., 2000; Seymour et al., 2002), may be higher in certain cultivars, thus resulting in a less strong fruit exocarp.

However, most strikingly, the CM inevitably covered a large range of the mechanics of the skin, at least at lower strains, and thus mirrored the mechanical properties of the FS. It can also be concluded from the transition points (Table 2) that the tomato fruit cuticle inherits a large contribution to the mechanical demands up to 3–5% total strain. At these strain levels, the mechanical behaviour and properties of the FS, in particular the modulus of elasticity, seemed to be determined to a high degree by the cuticle, whereas at higher levels they were governed mostly by the cell layers beneath. Under physiological conditions, which may fall into the 3–5% total strain, it is assumed that skin tensile resistance is more important than stress or strain at failure, which are only likely to be relevant under exceptional conditions. Consequently, the mechanical importance of the tomato fruit cuticle as an integrative structural component becomes evident. The same observations were made in the preliminary study, although with different absolute values (Fig. 7). In this study, the FS was tested fresh, whereas the isolation of the cuticle followed a similar protocol. A similar trend of ripening-related increase in stiffness of both cuticle and fruit skin can be depicted. Again, the cuticle mirrored the mechanical properties of the skin to a high degree. Despite this, there were considerable differences between the behaviour of the FS found in the preliminary study and the ‘main’ study that may primarily result from some preparation effects on the cell wall, whereas the mechanics of the cuticle was virtually identical. In the preliminary study, cell metabolic influences as well as changes in turgor during the course of mechanical testing, i.e. mechanical stress, could have directly affected the physical wall properties, resulting in a generally higher stiffness of the FS. It has been shown that water stress by a decreased water potential in the cell wall of rye coleoptiles (Secale cereale L.) caused a decrease in extensibility (Edelmann, 1995a). The possible effect of physiological stress reactions during testing has been eliminated in the ‘main’ experiments by incubation in ethanol, and, moreover, cytoplasmic solutes are supposed to have been thoroughly extracted during the washing procedure and incubation in the storage medium. Considering possible artefacts by ethanol on the cell wall physical properties, Edelmann (1995b) has reported a stiffening effect of ethanol/water solutions on rye coleoptiles, but this effect was fully reversible upon washing with distilled water, and thus, the preparation used for the FS is believed not to have significantly affected the mechanical properties of the cell walls.

Ripening-related changes of the mechanical properties of the CM

The observed ripening-related changes of the mechanical properties of the CM seem to be determined mainly by modifications of the chemical composition of the CM. In their study, Baker et al. (1982) showed that the amount of C18 tri-hydroxy-fatty acids decreased from immature to mature fruits, which would give rise to a relative gain of C16 cutin monomers in the cuticle of tomato fruit at stage nr. More recently, Marga et al. (2001) have undertaken chemical analysis of cuticles from different organs of a thistle (Cirsium horridulum Michx.), each having different biomechanical properties, and were able to correlate these properties with the predominant cutin monomer type, i.e. rigid cuticles can be classified by C16 cutin monomers, while more elastic cuticles correspond to mixed C16/C18. Thus, hydroxyl groups may enhance the hydrophilic character and the hydration state of the cutin matrix, which would result in a higher elasticity at immaturity. Graca et al. (2002) have also characterized tomato fruit cutin from ripe fruits as a C16 type, and reported small amounts of glycerol as an esterified constituent in the cutin-matrix that possibly reinforces the polymeric structure. Whether glycerol is abundant only in the case of maturity or also at
immature stage remains to be established. Moreover, abundance and increase of phenolic compounds in the cutin matrix of the CM during tomato fruit ripening, for example, 1-p-coumaric acid as well as the flavonoids naringenin and chalconaringenin, has been reported by several authors (Hunt and Baker, 1980; Baker et al., 1982; Laguna et al., 1999). The presence of ‘lignin-like’ phenolics in the tomato fruit exocarp has been speculated to be involved in the regulation of fruit growth (Andrews et al., 2002a, b). Available data indicate that the phenolic constituents form molecular clusters and/or bind covalently to cutin (Riley and Kolattukudy, 1975; Laguna et al., 1999), most reasonably at free available secondary hydroxyl groups proposed to be only partly esterified (Deas and Holloway, 1977; Heredia, 2003). Given this, the increase of flavonoids in the cutin network during ripening seems to reduce the flexural rigidity of the cuticle, either by reducing free available hydroxyl groups in the cutin matrix, or by a decrease of the free volume necessary for motional constraints being in a molecular domain of a mobile category in the membrane.

The observed increase of cutinization of the CM during ripening as a possible structural stiffening factor need not necessarily be involved in the reported changing mechanical properties, as has been proposed by Matas et al. (2004). Although it might be plausible that a sponge-like structure of the cutinized CM at later stages of maturity stabilizes the cuticle mechanically, the corresponding mechanical properties at stage mg did not indicate such a structural support. More plausible would be a stiffening influence on the CM by an increase in the cell wall polysaccharide content as reported for the tomato fruit cuticle (Baker et al., 1982).

Differences in cell wall content of the cuticle have been proposed to affect the mechanical performance of fruit exocarp of ripe tomato fruits (Matas et al., 2004). Biologically even more important, cutinization of cell walls could result in a fruit exocarp composite with the potential to be stronger than either component individually. This could be due to structural, but also chemical causes, since non-covalent interactions between cell wall polysaccharides are likely to be strengthened in the non-polar environment of the cuticle. In addition, tissue tension exerted on the outer fruit coverage may also account for the ripening-related mechanical changes, since this prestress could restrict the extensibility of the CM as well as the FS, resulting in higher stiffness.

A putative model of tomato fruit growth

From the results presented here, a generalized putative model of tomato fruit growth is proposed. Since rapid fruit expansion is predominant in the earlier stages of growth (Monseline et al., 1978; Thompson et al., 1998), equivalent to stage ig, the outer fruit coverage has to adapt to this expansion with a more ductile nature. As far as the cuticle is concerned, this is consistent with the majority of CM samples of immature fruits, which were characterized by strain softening or linear behaviour and high extensibility. A comparable low modulus of elasticity and high extensibility was revealed for both CM and FS. Strain softening biphasic behaviour has been reported for other plant material during early ontogeny, including Aristolochia stems (Köhler et al., 2000), whereas strain hardening material behaviour as displayed by FS throughout growth and maturation is more typical for the fibrous polymer network of cell walls (Vincent, 1990a, b; Niklas, 1992). At the mature-green ripening stage, equivalent to the phase where fruit growth declines, the material behaviour of the cuticle changed towards strain hardening uniformly for all three tomato fruit cultivars. Thus, extensibility may be restricted even at the apparently higher strains. At the fully ripe stage, stress–strain behaviour of the CM again underwent changes, but no uniform pattern could be observed. The overall predominant curve form was linear material behaviour, but strain softening and strain hardening also occurred. At this stage, fruit growth generally ceases, and coinciding higher tensile stiffness for both CM and FS for all three cultivars could be observed. Thus, the tensile measurements reported here indicate that the fruit exocarp provides a mechanical constraint to turgor-driven growth, while the cuticle inherits a large contribution to the overall tensile resistance. The described changes in material behaviour and properties agree well with the altered chemical composition and physiological modifications of the fruit exocarp as shown.

Conclusion

During tomato fruit growth and ripening, morphology and the mechanical properties of the cuticle as well as the epidermis were subject to considerable changes. Pronounced cultivar-specific differences could be also noted. The mechanical properties of the FS exceeded that of the CM, but were largely mirrored by the biopolyester membrane at all ripening stages of the three cultivars tested. Thus, evidence is high for the cuticle to become increasingly important as a structural component for the integrity of the ripening tomato fruit. It is even possible to speculate that the cuticle impacts on morphogenetic processes, and may be involved in the determination of fruit growth rate in addition to the epidermis, since the mechanisms responsible for fruit growth regulation are likely to be multiple. The results are also of vital interest for breeders and the fruit industry, since the reported mechanical properties, in particular the decrease of extensibility of the tomato fruit cuticle at the final stages of ripening, also have implications concerning cracking susceptibility, harvesting, transportation, and extended storage. Future investigations are required to analyse further the relationship between the mechanical properties and the dynamic changes of the chemical composition of the tomato fruit cuticle throughout maturation, as well as comparative studies of other cultivars.
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