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Viscoelastic-plastic behavior of single tomato mesocarp cells in high speed compression-holding tests

Zhiguo Li[1,2,*], Zhibing Zhang[1], Colin Thomas[1]

[1]*School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*

[2]*School of Mechanical and Power Engineering, Henan Polytechnic University, 454003 Jiaozuo, China*

[*]Corresponding author. Tel.: 44-121-414-5081; Fax: 44-121-414-5324. E-mail: lizhiguo0821@163.com

Abstract

The micromechanics of isolated tomato fruit cells were investigated by microcompression-holding. Covering the cells with deionized water after isolation caused no significant volume changes, suggesting that cells suspended in water for compression testing were representative of those in the original tissue. The viscoelastic-plastic behavior of such cells was characterized by compression at 4900 ± 200 μm s^{-1}, then holding. Although the cells were generally not spherical initially and some cell deformation appeared to be local, the force-time data were fitted by the Hertz-Maxwell model for relaxation of viscoelastic spheres. The force at 15% deformation, instantaneous and equilibrium elastic moduli, yield strength, and first and second relaxation times were 2.5 ± 0.6 mN, 0.6 ± 0.3 MPa, 0.22 ± 0.08 MPa, 0.03 ± 0.01 MPa, 0.48 ± 0.05 s, 0.033 ± 0.004 s, respectively. These parameters showed little sensitivity to several reasonable definitions of cell size nor to changes in the (assumed) Poisson's ratio.

Industrial Relevance*:* Fresh fruit is very susceptible to damage during industrial handling (e.g. mechanical harvesting, packaging and transport). Mechanical damage to fruit, manifested at the macro scale, is caused ultimately by failure of cells at the micro scale. Viscoelastic-plastic characterization of single cells isolated from tissue is vital to macro-scale modelling, simulation and prediction of mechanical damage to fruits. The method might be extended to single cells of other fruits.

Keywords: Tomato fruit; Single cells; High speed compression; Viscoelastic-plastic; Hertz-Maxwell model

1. Introduction

Tomato fruits are an important component of many human diets and therefore their quality is also important. Unfortunately, fresh fruit is very susceptible to mechanical damage caused by any rapid impact during harvesting, packaging and transport, and the quality can be substantially reduced by poor handling (Li & Thomas, 2015a). Damage may lead to accelerated rot of a whole fruit, which is both a food safety

and an economic issue. Mechanical damage to fruit, manifested at the macro scale, is caused ultimately by failure of cells at the micro scale (Li & Thomas, 2015b). Investigation of the viscoelastic-plastic behavior of single cells is necessary to macro-scale modelling, simulation and prediction of mechanical damage to fruits (Li, 2013).

In previous work, the elastic properties of single tomato suspension cells or fruit cells have been investigated by micro-compression at speeds of 6, 23, 43, 1500 μm s^{-1} to a percentage deformation of 20-40% (Blewett, Burrows, & Thomas, 2000; Wang, Pritchard, & Thomas, 2006; Wang, Wang, & Thomas, 2004) and micro-puncture at a speed of 20 μm s^{-1} to a deformation of 10 μm (Zdunek & Kurenda, 2013). The plastic properties of tomato fruit cells have been investigated in part by macro-puncture of tissues and deduction of the mechanical properties by modelling (Li, Lv, Wang, Zhao, & Yang, 2015). There has also been research on the mechanical properties of some cell wall components of tomato fruit tissues, such as cellulose and xyloglucan, by small deformation oscillatory rheology and uniaxial tensile testing for determining the shear modulus (Whitney, Gothard, Mitchell, & Gidley, 1999). The elastic-plastic properties of cuticular membrane have been determined by one-dimensional tension testing (Bargel & Neinhuis, 2005; Matas, Lopez-Casado, Cuartero, & Heredia, 2005) and those of tissues by compression, tension, shear and bend tests (Li, Li, Yang, & Liu, 2013; Li, Li, Yang, Liu, & Xu, 2012). Other related research focused on the elastic-plastic properties of the plant cell wall by relaxation spectra or dynamic nanoindentation (Hansen *et al.*, 2011; Hayot, Forouzesh, Goel, Avramova, & Turner, 2012).

Despite all these studies, very little information is available on the viscoelastic-plastic properties of single fruit cells, especially at high compression speeds that mimic the effects of rapid impacts on fruits. Because the speeds of such impacts during mechanical handling are often more than 1000 μm s^{-1} (Li & Thomas, 2015a), it is difficult without single cell compression testing at such speeds (or higher) to create useful multi-scale mathematical (finite element) models for linking and simulating the macroscopic (whole fruit or organ) scale to the microscopic (cellular) scale. Additionally, single cells recovered from tissues for compression testing have previously been suspended in solutions of chosen osmotic pressure (Blewett *et al.*, 2000; Wang *et al*., 2004) without any knowledge of whether their volume was changed from tissue to suspension. The objective of this study was therefore i) to check whether the volume of single tomato fruit cells recovered from tissues changed or not after their suspension in deionized water; ii) to characterize the viscoelastic-plastic behavior of single cells from tomato tissues by micro-compression testing at high speed, followed by holding to allow determination of relaxation parameters.

2. Materials and methods

The experiments were conducted at the University of Birmingham, United Kingdom. Fresh-market *Elegance* vine tomatoes (Thanet Earth company, UK) were bought from a supermarket in April 2015. These fruits were inspected to ensure that they were not damaged or infested with insects prior to transport to the laboratory. Then the fruit surfaces were manually cleaned with water and dried before storage in a refrigerator at about 4^{o}C before samples were prepared for testing. One package of 5 tomatoes was used for all samples. Preliminary work has shown no significant differences in the mechanical behavior, characterized by firmness, of tomatoes taken from a single vine (data not shown).

2.1 Test of volume changes of single tomato fruit cells on suspension in deionized water

Mesocarp tissue of excised pericarp blocks was quickly brushed using a test tube brush, to isolate and transfer single cells onto a glass slide. The slide was placed immediately on the stage of a light microscope (Leica DMRBE, Spectra Services, Inc., USA) and deionized water was gently dropped onto the cells to cover them. An electronic eyepiece (Moticam Pro 252B, Motic Deutschland GmbH, Germany) was used to photograph the cells before and after covering with deionized water. Images of the cells were selected before adding water (0 s, original cells), and then at 5, 1800 and 3600 s after wetting. The projected areas of the same cells on each slide were found using Motic Images Plus 2.0 software. In total, ten cells were tested. The projected area was regarded as a surrogate parameter for the volume of the cells.

2.2 High speed microcompression-holding tests

2.2.1 Preparation of single cells

In order to obtain single cells from tomato tissues for compression testing, each tomato was cut into quarters and then some rectangular pericarp blocks (length × width: 50 mm × 10 mm) were prepared with the endocarp removed. As shown in Fig. 1, the inside of each pericarp block was quickly brushed using a test tube brush to isolate and transfer single cells of mesocarp tissue into deionized water in a small beaker. Lastly, some of the cell suspension was transferred from the beaker into a glass chamber using a 3 mL dropper (Fig. 1). Some single cells that were too close together were gently separated using air blown from a dropper. During testing, the suspension in the beaker was replaced every half an hour to avoid significant physiological changes to samples during testing.

2.2.2 Microcompression tester

The basic method used in compression-holding tests of single cells has been described by Wang *et al*. (2004, 2006). As shown in Fig. 2, the glass chamber mentioned earlier containing single cells was put on

the chamber holder under an inverted microscope. Single cells were positioned accurately underneath the glass compression probe by simultaneous use of the side and bottom view cameras. The probe was fixed to a force transducer (Model 406A, Aurora Scientific Inc., Canada) and both were moved down to make the flat end of the probe level with the top of a chosen single cell in the chamber. This allowed the cell to be compressed between the probe and the bottom surface of the chamber. Force-time and force-deformation data were measured to a final deformation of 15%, for nineteen cells within 10 hours. In order to minimise time-dependent effects during compression such as viscoelasticity and possible loss of water from the protoplast, the cells were compressed at a high speed i.e. 4900 ± 200 μm s^{-1} (approximately the limiting speed of the tester) by a piezo-stack (P-841.60, Physik Instrumente (PI) GmbH & Co. KG, Germany) with a 90 μm maximum movement. After compression each cell was held at constant deformation for a short time. As the piezo-stack was fixed to the base of the micromanipulation equipment, and the chamber holding the suspension of single cells was placed on top of the stack, the cells were actually compressed against the force transducer probe by upward displacement of the piezo-stack and chamber (Wang, Cowen, Zhang, & Thomas, 2005) rather than downward motion of the probe. The compression speed was an average value, i.e. the probe displacement during cell compression divided by the time of motion taken from transducer data.

2.3 Extraction of physical and mechanical parameters

2.3.1 Physical parameters

Because the cells were irregular in shape and not specifically aligned to the camera views, the apparent shape of the cells in the side view approximated a circle while the bottom view always approximated an ellipse. Therefore, the key dimensions of the cells were measured before and after compression in order to investigate their effect on the mechanical properties, and to discover the effect of compression and relaxation on the geometry. Before compression, the height H_1 and the length of the major and minor axes (of the cross-section in the bottom view) L_1 and W_1 of each cell were measured by digital caliper from the side view in monitor 1 and the bottom view in monitor 2, respectively (Fig. 1). After compression-holding, the corresponding cell sizes H_2, W_2 and L_2 were also measured. Subsequently, some geometrical parameters such as sphericity, geometric mean diameter and percentage change were calculated using equations (1-3). The geometric mean diameter is an expression of the average size of irregular objects often used in Agriculture Science (Haciseferogullari, Gezer, Ozcan, & MuratAsma, 2007; Li, Li, & Liu, 2011; Li & Thomas, 2014).

$$GMD_{1\text{or }2} = \sqrt[3]{H_{1\text{ or }2} \times W_{1\text{ or }2} \times L_{1\text{ or }2}} \quad (1)$$

$$\varphi = \frac{GMD_1}{MAX(H_1,\ W_1,\ L_1)} \quad (2)$$

$$r_{GMD} = \frac{GMD_1 - GMD_2}{GMD_1} \times 100\% \quad (3)$$

where $GMD_{1\text{ or }2}$- geometric mean diameter of the cell before or after compression-holding, $H_{1\text{ or }2}$ - cell height before or after compression-holding, $W_{1\text{ or }2}$– length of the minor axis of the cell before or after compression-holding, $L_{1\text{ or }2}$ – length of the major axis of the cell before or after compression-holding, φ – sphericity of cell, r_{GMD} –percentage change of geometric mean diameter after compression-holding relative to that before compression.

2.3.2 Viscoelastic-plastic parameters

During multiscale modelling of a fruit using finite element simulation, the number and complexity of the elements representing the cells are key factors. A 50 mm diameter tomato fruit contains more than 2.9×10^6 cells if their sizes range from 250 to 350 μm. One approach to modelling cells is to regard them as equilibrium homogeneous bodies, so that a finite element model of tomato fruit including many cells can be simplified to make simulations practical. Therefore, in this study the cells were assumed to be homogeneous solids (Fig.3) and the corresponding viscoelastic-plastic properties were found. The implication of this approach is that any relaxation on holding a compressed cell is assumed not to depend on water flows from the protoplast but to be due to viscoelasticity. The cells were treated in the analysis as if they were spherical.

Firstly, elastic-plastic parameters of the cells i.e. the force at 15% deformation, referred to here as "peak force" F_{max}, the apparent elastic modulus in compression E_a and the yield strength σ_y, were derived by fitting the Hertz contact model i.e. equations (4-6) (Kogut & Etsion, 2002; Wang, et al., 2005) to force-displacement data for each cell during compression. Subsequently, the force-time data during force-relaxation on holding of a compressed cell were fitted by some general Maxwell models of increasing complexity. The Maxwell model which includes 3 spring and 2 dashpot elements, as also used by Yan, Zhang, Stokes, Zhou, Ma, & Adams (2009), was finally regarded as suitable to characterize the viscoelastic properties of the cells (Fig.3). Finally, the viscoelastic mechanics of each cell, which was characterized by the equilibrium elastic modulus E_∞, instantaneous elastic modulus E_0 and relaxation times τ_1, τ_2, were derived by the Hertz-Maxwell extension model represented by equations (7-9) (Chen, 2014; Yan, Zhang, Stokes, Zhou, Ma, & Adams, 2009).

$$\varepsilon = \frac{D}{H_1} \times 100\% \quad (4)$$

$$\mathrm{F} = \frac{\sqrt{2R}E_a}{3(1-\mu^2)} D^{1.5} \quad (5)$$

$$\sigma_y = \frac{1}{6.22(0.45+0.41\mu)(1-\mu^2)} E_\infty (\frac{D_0}{R})^{1/2} \quad (6)$$

$$\mathrm{F}(t) = \frac{2\sqrt{2R}}{3(1-\mu)} D_1^{1.5} G(t) = \frac{2\sqrt{2R}}{3(1-\mu)} D_1^{1.5} [G_\infty + G_1 \exp\left(-\frac{t}{\tau_1}\right) + G_2 \exp\left(-\frac{t}{\tau_2}\right)] \quad (7)$$

$$E_\infty = 2G_\infty(1+\mu) \quad (8)$$

$$E_0 = 2G_0(1+\mu) = 2\left(G_\infty + G_1 + G_2\right)(1+\mu) \quad (9)$$

where H_1 - height of cell before compression, μm; D – cell deformation (probe displacement), μm; ε – percentage deformation, %; F – applied force during compression, mN; E_a - apparent elastic modulus in compression, MPa; F(t) - instantaneous force during holding, mN; G(t) - shear modulus of cell, MPa; D_1 – cell deformation at 15%, μm; D_0 – cell deformation at 11%, μm; G_∞ - equilibrium shear modulus of cell, MPa; G_0 - instantaneous shear modulus of cell, MPa; G_i - shear modulus of i^{th} element, MPa; τ_1, τ_2 - relaxation time of the 1st and 2nd Maxwell element, *s*; E_∞ - equilibrium elastic modulus, MPa; E_0 - instantaneous elastic modulus, MPa; μ – Poisson ratio; R – initial radius of cell, μm; σ_y– yield strength, MPa.

2.4 Statistical analysis

The experimental results were analyzed for statistical significance using variance analysis in SAS 9.2 software (SAS Institute Inc., USA), with a significance level of $\alpha = 0.05$, and for least-square fitting of the Maxwell model using nonlinear regression analysis in SigmaPlot 12.5 software (Systat Software Inc., UK). All the values listed in this paper are mean ± 95% confidence interval.

3. Results and discussion

3.1 Volume changes of single tomato fruit cells on suspension in deionized water

The four stages of single tomato cells covered by deionized water for 0, 5, 1800 and 3600 s are presented in Fig. 4. The mean projected areas of ten cells at each stage were 0.14 ± 0.04, 0.13 ± 0.03, 0.16 ± 0.04, 0.13 ± 0.03 mm^2, respectively. The variance analysis showed that there was no significant change in the mean projected area across the four stages. By assumption therefore, there was no significant difference in cell volume before and after covering the cells with deionized water. As the cells at 0 s were not yet covered in water, this implies that the osmotic environment of the cells in the tissues was like water or at least was so dilute that subsequent suspension in deionized water did not cause observable volume

change. Any effects of the cells being suspended in distilled water on measurements of cell mechanic properties were therefore discounted. The most useful conclusion of this observation is that microcompression testing of tomato cells suspended in deionized water can be regarded as equivalent to testing cells in the environment they were originally in the mesocarp.

3.2 Typical compression-holding curves

Fig. 5a is a force-time trace for the most common compression-holding behaviour shown by single tomato fruit cells. Because of the high speed compression, the loading time during compression was very short (11.9 ms) and the compression force increased very quickly until it reached peak force at the chosen final deformation of 15%. The force-displacement data during compression were used in elastic-plastic analysis of single cells. From the peak force, during holding, the compression force started to decay with time. In this phase, the cell deformation (displacement of the Piezo-stack) was a constant. The decay suggested a stress relaxation process. The force-time data in this phase were also recorded and were used to analyse the viscoelasticity of the cells. For the purposes of modelling the cells for future finite element analysis, it was assumed the cells were not losing water from the protoplast, which would also have led to stress relaxation. Ideally one would test this assumption by measuring volume changes during relaxation but the changes were too small to be determined reliably.

The typical force-percentage deformation and force-displacement data corresponding to the compression phase of Fig. 5a are showed in Fig. 5b and 5c. As there is no sudden drop in the force, as observed for example by Blewett *et al.* (2000), it is clear that this cell (and in fact all the cells) had not burst before the chosen final deformation of 15%, nor had there been a catastrophic failure of the plasmalemma during compression. Following holding, when the probe was moved away from the cell (by lowering the chamber), permanent deformation of the cell was clearly visible in the side view and there was no obvious recovery. This suggests that 15% deformation was above the elastic limit and plastic deformation had occurred. The red points on the curves in Fig. 5b represent the elastic limit value of 11% measured directly by Wang *et al.* (2006). Therefore, the apparent elastic modulus and yield strength in the compression phase were derived from percentage deformation data of <11%.

3.3 Geometrical characteristics of single tomato cells

The mean height and mean initial geometric mean diameter of the cells were 400 ± 35 μm and 460 ± 30 μm respectively. Fig. 6a is a frequency distribution histogram of the sphericity of single tomato fruit cells obtained by the mechanical isolation method of this experiment. The sphericity ranged from 0.58 to

0.95 and the sphericity of 74% of the cells (yellow + light green + dark green in Fig. 6a) was less than 0.8 (Fig. 6b), which illustrated that most of the cells from tomato mesocarp tissues were of irregular shape rather than spherical. Because of this lack of sphericity, it was important to consider the sensitivity of the Hertz-Maxwell model to the geometry of the cells, as will be discussed in section 3.4. Fig. 6c shows that there was a strong linear correlation between initial geometric mean diameter GMD_1 and initial height H_1 of the cells ($p<0.05$). The geometric mean diameters before and after compression-holding, GMD_1 and GMD_2 respectively, and the relative change RC_{GMD} are shown in Fig. 6d. There was a significant difference ($p<0.05$) of – 4.5 ± 0.3% in the geometric mean diameter of cells before and after compression-holding. The relative change in height was the deformation i.e. – 15% and the lengths of the major and minor axes during compression had only small changes. This suggested the deformation of cells was local along the compression direction, probably because of plastic deformation due to the high deformations at very high speeds.

3.4 Effect of cell geometry on viscoelastic-plastic properties

3.4.1 Sensitivity of the Hertz-Maxwell extension model to Poisson's ratio and the chosen cell radius

For equations (5-8), the instantaneous elastic modulus E_0, equilibrium elastic modulus E_∞ and yield strength σ_y were obtained by inputting the cell radius R and Poisson's ratio μ. However, the Hertz-Maxwell extension model assumes a cell shape of sphere, and in approximating non-spherical cells to spheres, several values of R might have been chosen i.e. half of height H_1, half of the geometric mean diameter GMD_1 or half the length of the major axis L_1. The sensitivity of the mechanical parameters E_0, E_∞ and σ_y to these choices was investigated, as was the effect of changing the assumed Poisson's ratio between 0.2 and 0.49, based on the description of Brizmer, Kligerman, and Etsion (2006) and Burubai, Amula, Etekpe, Alagoa, Preye, and Suoware (2009). The Poisson ratio is unknown and a value of 0.4 was chosen (Wang *et al.*, 2006). Even if μ was chosen to be 0.2 (surely an extreme choice) the parameter estimates would not change more than 10% and given the measurement errors, this is not significant. It appeared that the choice of R had no statistically significant effect (Fig. 7). Further, H_1 can be regarded as a better choice than GMD_1 because it only requires one measurement on the original cell. This shows that the Hertz-Maxwell extension model can be used to investigate the viscoelastic properties of single tomato cells by inputting an assumed Poisson's ratio μ of 0.4 and choosing the cell radius R to be $H_1/2$ (which is linearly dependent on GMD_1, according to Fig. 6c). A similar finding of the effect of Poisson's ratio changes was reported by Wang *et al.* (2004) and Dintwa *et al.* (2011). It was inferred that it is valid to apply the

Hertz-Maxwell extension model to irregularly shaped but approximately spherical cells.

3.4.2 Viscoelastic-plastic properties of single tomato cells

The peak compression force, the instantaneous elastic modulus, the equilibrium elastic modulus, the yield strength, the first relaxation time and the second relaxation time of single tomato cells under compression at a very high speed of 4900 ± 200 μm s^{-1} were 2.5 ± 0.6 mN, 0.6 ± 0.3 MPa, 0.22 ± 0.08 MPa, 0.03 ± 0.01 MPa, 0.48 ± 0.05 s, 0.033 ± 0.004 s, respectively. This compression speed has never before been employed in this application and this is important when considering the possible speeds of impacts on tomato fruits. The mean apparent elastic modulus in high speed compression of the cells was 0.8 ± 0.2 MPa. Statistically, the instantaneous elastic modulus was not significantly different from the apparent elastic modulus in compression. This is attributed to the very high speed in compression, which makes any viscoelastic effects negligible in this phase. (If the compression speed had been extremely low, as in some earlier studies, the apparent elastic modulus in compression might be expected to be close to the equilibrium elastic modulus.) Previous micro-compression testing of single tomato fruit cells in 0.03 M mannitol solution showed that the compression force at 15% deformation of such cells was ca. 0.2 mN at a compression speed of 1500 μm s^{-1} (Wang *et al.*, 2006). In another determination of the elastic modulus of tomato fruit cells by atomic force microscopy, values of 100 kPa and 20 kPa were obtained when the probe tip was sharp or a bead tip, respectively (Zdunek *et al.*, 2013). Even leaving aside any effects of high external osmotic pressure on the size and mechanical behaviour of tomato cells, one should expect significantly differences between the cell wall moduli determined in earlier work and the whole body moduli found here. Furthermore, the equilibrium elastic modulus E_∞ measured in this study was 2.2 times and 11 times higher than that measured by atomic force microscopy with a sharp tip and a bead tip, respectively. This difference might be attributable to (i) the cell samples were from different tomato cultivars and tissues, (ii) the cell samples were placed in different solutions during compression and (iii) the cell samples were compressed to different deformation level during testing. However, it should also be noted that the atomic force microscope method has been criticised for measuring only local and surface properties, giving lower moduli *(Haciseferogullari et al., 2007; Zdunek et al.*, 2013) and testing cells embedded in tissue (Rodriguez, McGarry, & Sniadecki, 2013) may also give misleading results due to cell-cell adhesion.

Although the peak force F_{max} at 15% deformation measured here was approximately the same as the bursting force of single suspension-cultured tomato cells (Blewett et al., 2000) none of the nineteen single

tomato fruit cells were obviously broken, based on direct observations. It is likely that the bursting behaviour of cells isolated from tomato fruits is different from that of suspension-cultured cells and that a percentage deformation of 15% is not adequate to burst the former. Under 15% deformation, the forces needed to compress single tomato fruit cells at a compression speed of 4900 μm s^{-1} (more than 3 times the 1500 μm s^{-1} used by Wang *et al.* (2006)) were about 12 times higher than those applied to suspension-cultured tomato cells by Wang *et al.* (2004). It is possible the lower speed allowed some water flows from the suspension-cultured tomato cells but it is also likely suspension-cultured and isolated fruit cells have different wall composition and structures. There is no information about the yield strength and Maxwell relaxation time of single tomato cells in the literature so no comparisons are possible.

4. Conclusions

Viscoelastic-plastic characterization of single cells is important to macro-scale modelling and simulation of mechanical damage to fruits and vegetables. In this study, this was achieved by compression of single cells recovered from tomato mesocarp tissues at very high speeds (4900 ± 200 μm s^{-1} to a final percentage deformation of 15%), with subsequent relaxation at constant deformation. The force-time data were recorded and used to analyse the viscoelasticity of the cells. It was assumed the cells were not losing water from the protoplast, which would also have led to stress relaxation and that they could therefore be treated as homogeneous bodies. This approach will be useful for the purposes of modelling the cells in future finite element analysis.

There was no significant difference in cell volume before and after covering the cells with deionized water. This implies that the osmotic environment of the cells in the tissues was water-like. The conclusion is that microcompression testing of tomato cells suspended in deionized water can be regarded as equivalent to testing cells still in the mesocarp.

Nearly all of the cells were of irregular shapes but the Hertz-Maxwell extension model showed little sensitivity to the choice of parameter describing cell “radius”. Therefore, the Hertz-Maxwell extension model can be used to investigate the viscoelastic-plastic properties of irregularly shaped single tomato cells and this simplification will be of practical use in finite element modelling as the alternative would be very complex and computationally expensive.

The peak compression force, the instantaneous elastic modulus, the equilibrium elastic modulus, the yield strength, the first relaxation time and the second relaxation time of single tomato cells were 2.5 ± 0.6 mN, 0.6 ± 0.3 MPa, 0.22 ± 0.08 MPa, 0.03 ± 0.01 MPa, 0.48 ± 0.05 s, 0.033 ± 0.004 s, respectively. These

cell mechanical parameters should be useful in modelling the relationship between macro-scale and micro-scale mechanics of tomato fruits and it should be possible to extend the method to other fruits provided single cells can be isolated from tissues.

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Figures

Figure 1

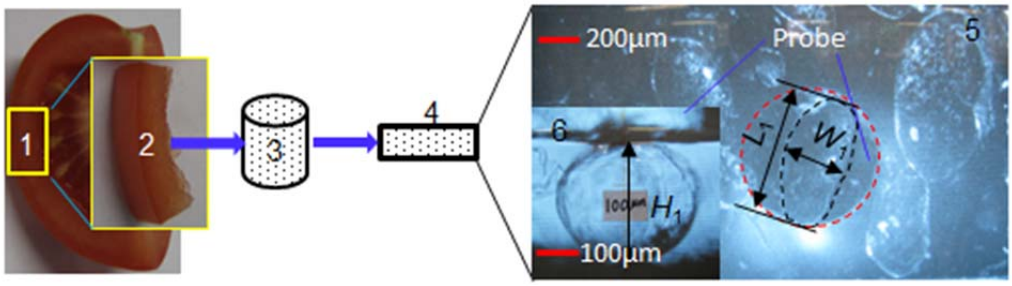

Fig. 1 Preparation of single cells. 1-pericarp wall, 2-pericarp block without endocarp, 3-beaker, 4-chamber, 5 - bottom view of chamber with some isolated single cells, 6-side view, W_1-length of minor axis, L_1- length of major axis, H_1-initial height.

Figure 2

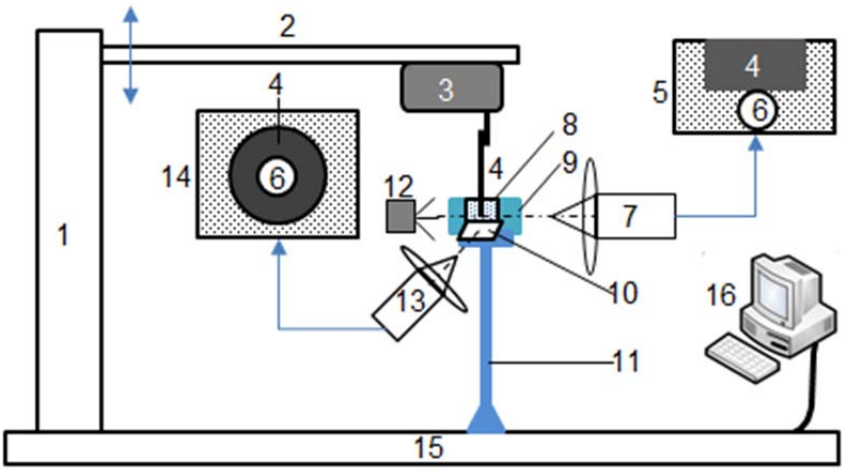

Fig. 2 Microcompression tester. 1-carriage and lead rail, 2-cantilever, 3-force transducer, 4-glass probe, 5-monitor 1, 6-single cell, 7-P-CAM camera, 8-glass chamber, 9-chamber holder, 10-mirror at 45 degrees, 11-piezo-stack, 12-light source, 13-CCD camera, 14-monitor 2, 15-base,16-computer.

Figure 3

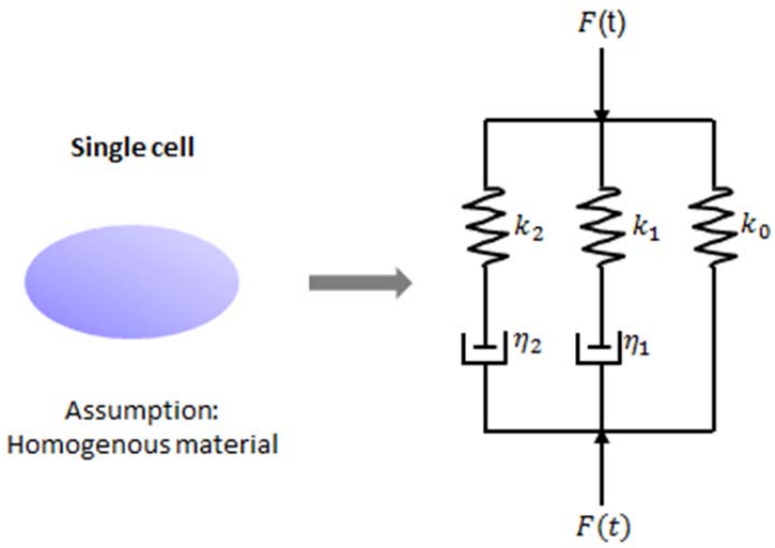

Fig. 3 Maxwell model including 3 spring and 2 dashpot elements. *F*(t) is the instantaneous force during holding; k_0, k_1 and k_2 are the linear elastic constants of the *first, second and third* springs respectively; η_1 and η_2 are the linear viscous constants of the *first and second* dashpots respectively.

Figure 4

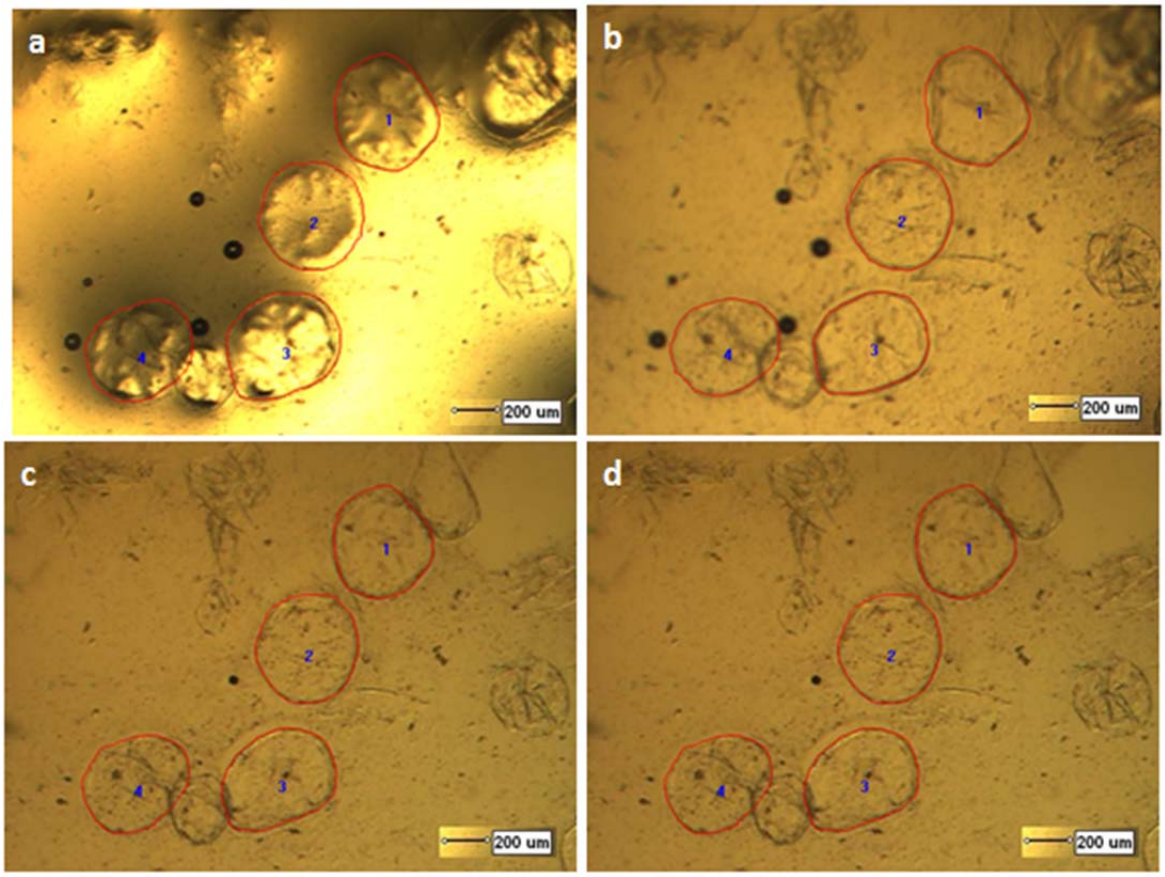

Fig.4 Volume change of single tomato cells before and after covering with deionized water. (a) Four cells before being covered by deionized water; (b) The same four cells covered by deionized water for 5 s; (c) for 1800 s; and (d) for 3600 s. The mean projected areas of the 1st, 2nd, 3rd and 4th cells were 0.15, 0.14, 0.16, 0.13 mm^2, respectively.

Figure 5

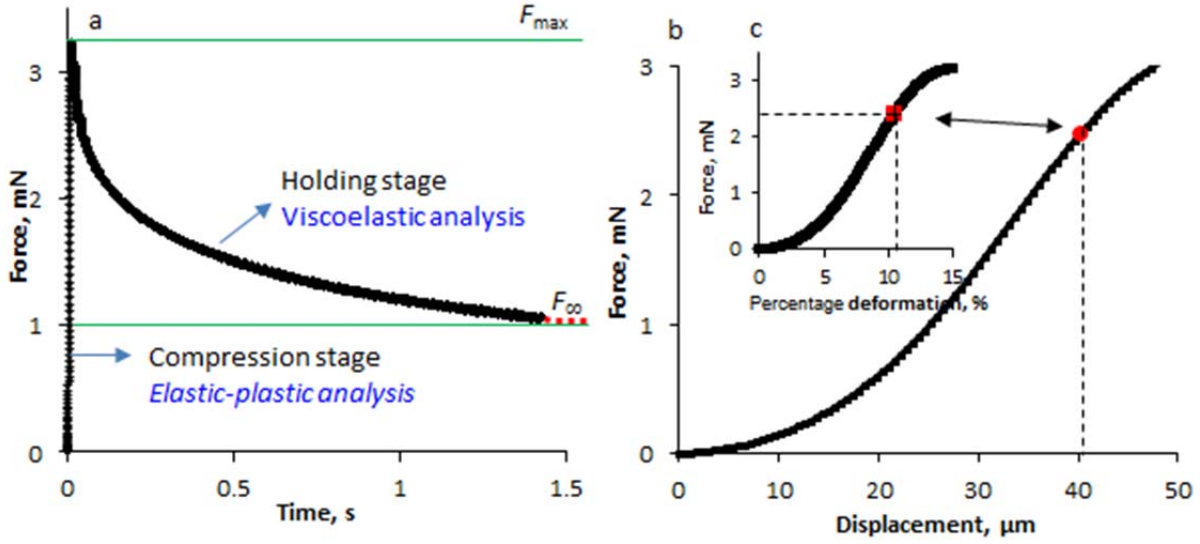

Fig. 5 A typical compression-holding curve. (a) Force-time curve, F_{max} is the peak force at 15%, F_∞ is the equilibrium force after holding, which is deduced from the fitted Maxwell model; (b) Force-displacement curve for compression only; (c) Force – percentage deformation curve. Cell height H_1: 380 µm, geometric mean diameter GMD_1: 458 µm, sphericity φ: 0.66, compression speed: 4800 µm s^{-1}.

Figure 6

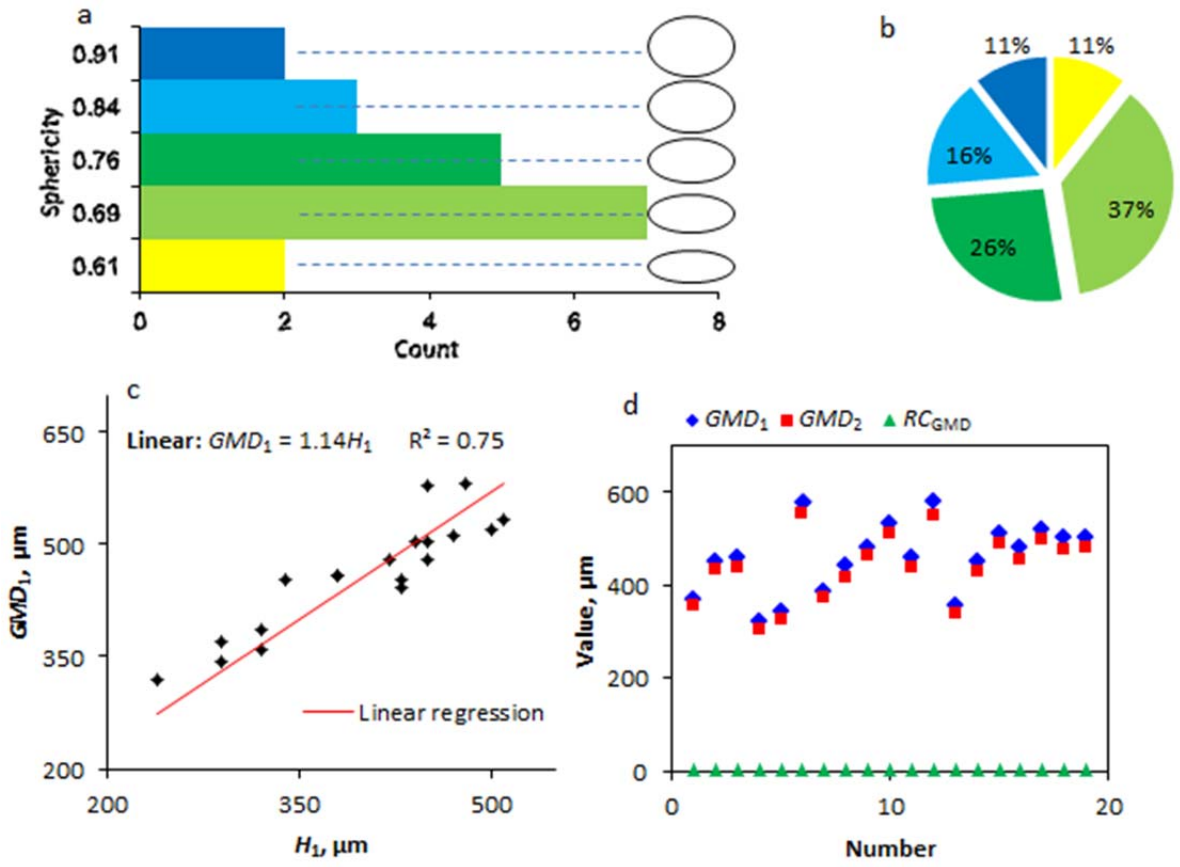

Fig. 6 Geometrical characteristics of single tomato cells. (a) Frequency distribution graph of sphericity of single cells, φ – sphericity; (b) Percentage of single cells within the 5 ranges of Fig. 6a; (c) Relationship between geometric mean diameter GMD_1 and initial height H_1 of cell before compression; (d) Geometric mean diameters GMD_1 and GMD_2 respectively, and percentage change RC_{GMD} before and after compression-holding.

Figure 7

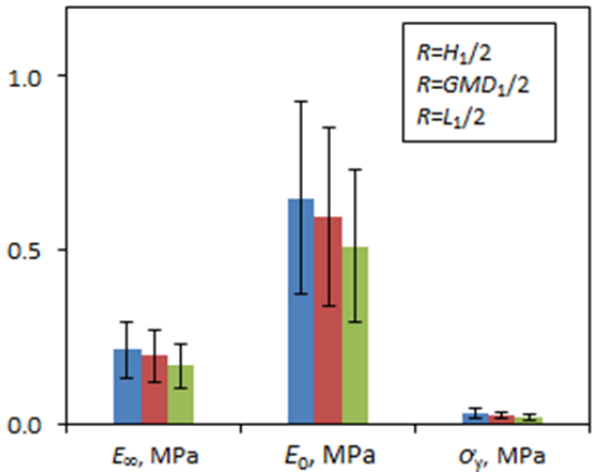

Fig.7 Sensitivity of the Hertz model to the choice of cell radius. Effect of choice of cell "radius" on the instantaneous elastic modulus E_0, the equilibrium elastic modulus E_∞ and the yield strength σ_y, H_1=340 ± 35 μm, GMD_1=460 ± 30 μm, L_1=620 ± 40 μm.