



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



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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 $4900 \pm 200 \mu\text{m s}^{-1}$, 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 $2.5 \pm 0.6 \text{ mN}$, $0.6 \pm 0.3 \text{ MPa}$, $0.22 \pm 0.08 \text{ MPa}$, $0.03 \pm 0.01 \text{ MPa}$, $0.48 \pm 0.05 \text{ s}$, and $0.033 \pm 0.004 \text{ s}$, 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 modeling, simulation and prediction of mechanical damage to fruits. The method might be extended to single cells of other fruits.

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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 modeling, 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, $1500 \mu\text{m s}^{-1}$ 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 $20 \mu\text{m s}^{-1}$ to a deformation of $10 \mu\text{m}$ (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 modeling (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 $1000 \mu\text{m s}^{-1}$ (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

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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; and 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 °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

The 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 \times width: 50 mm \times 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 the single cells has been described by Wang et al. (2004, 2006). As shown in Fig. 2, the glass chamber mentioned earlier containing the single cells was put on the chamber holder under an inverted microscope. The 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 h. In order to minimize 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. $4900 \pm 200 \mu\text{m s}^{-1}$ (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 Eqs. (1–3). The geometric mean diameter is an expression of the average size of irregular objects often used in

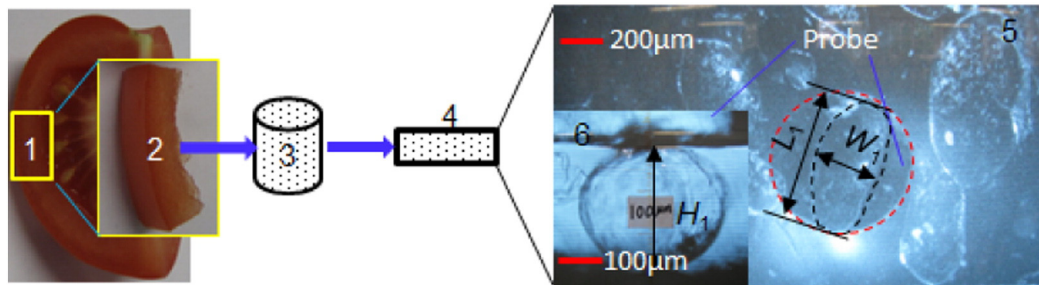


Fig. 1. Preparation of single cells. 1—pericarp wall, 2—pericarp block without endocarp, 3—beaker, 4—chamber, 5—bottom view of chamber with some isolated single cells, 6—side view, W_1 —length of minor axis, L_1 —length of major axis, and H_1 —initial height.

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