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Assessment of deformability of soft plant cells by 3D imaging

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ABSTRACT

Purees of fruit and vegetables are concentrated suspensions of plant particles. To elucidate the link between process and texture, it is necessary to understand their rheological behaviour, which is related to the plant cell volume fraction. Nonetheless, the concept of plant particle volume has yet to be clearly defined. From a physical point of view, plant cells can be considered soft, deformable, and compressible particles that tend to decrease their volume under stress or when the concentration increases, making the volume fraction difficult to determine. To the best of our knowledge, the ability of cells to compress themselves has never been shown directly in processed fruit and vegetables. Using confocal microscopy and three-dimensional (3D) reconstruction, we developed a protocol for determining the volume of cells in a concentrated suspension. Four suspensions varying in cell wall content were analysed. The cell volume decreased only in highly concentrated suspensions. We demonstrated by direct observation the ability of plant particles to compress and decrease their volume when the concentration of the suspension increases.

1. Introduction

Texture is a major attribute of food quality (Szczesniak & Kahn, 1971) and is defined as a sensory multi-parameter property that derives from the structure of a food (Szczesniak, 2002).

In apples, texture is the consumer's preferred sensory property (McAtee, Hallett, Johnston, & Schaffer, 2009), with the hedonic liking of apple texture being closely linked to crunchiness and juiciness (Allan-Wojtas, Sanford, McRae, & Carbyn, 2003). The cell number and size have been shown to be critical parameters for textural differences in apples: the cell number is important for predicting crunchiness and mealiness of the fruit, whereas cell size has been linked to juiciness (larger cells leading to juicier fruits) (Allan-Wojtas et al., 2003; Mann, Bedford, Luby, Vickers, & Tong, 2005; McAtee et al., 2009). Regarding processed apple products, Espinosa-Muñoz, Symoneaux, Renard, Biau, and Cuvelier (2012) have been able to link instrumental and sensory properties. They have shown that textural properties of apple purees can be dissociated in two principal sensory dimensions: 'consistency' and 'graininess'. These sensory properties have been shown to be related respectively to 'pulp content' and 'particle size'. As in raw apple, the cell number and size seem to be key parameters for the processed fruits' texture.

Texture of fruit and vegetable purees is closely linked to their rheology (Appelqvist, Cochet-Broch, Poelman, & Day, 2015; Espinosa et al., 2011; Moritaka, Sawamura, Kobayashi, Kitade, & Nagata, 2012). It is now well known that the rheology of fruit and vegetable purees is determined by the amount of cell walls and morphology of the particles. The quantity of cell walls and the volume they occupy in the continuous phase are responsible of the product's rheology. Insoluble solids content and cell morphology are thus key parameters for describing the texture of a puree (Day, Xu, Øiseth, Lundin, & Hemar, 2010; Espinosa-Muñoz, Renard, Symoneaux, Biau, & Cuvelier, 2013; Hemar, Lebreton, Xu, & Day, 2011; Moelants et al., 2014). Nevertheless, modelling rheological properties of purees to predict their texture remains difficult because assessment of the volume fraction of particles has not yet been achieved (Day et al., 2010; Lopez-Sanchez, Chapara, Schumm, & Farr, 2012). It is not easy to determine the volume fraction of the cells in such processed products, because once cooked, cells are widely deformable and able to compress under stress (Lopez-Sanchez & Farr, 2011). Many questions thus remain unanswered: At what concentration do cells become compacted? How do they compact? Is it possible to estimate the volume of cells in suspension? How can researchers assess the anisotropy of the deformation? There is growing interest in better measurements of structural parameters of such suspensions to predict the texture of the products.

Microscopy is widely used to examine fruit and vegetable cells, either raw or cooked, and can be applied to determine cell morphology. In 2009, McAtee et al. (2009) devised a quick method for determining the size and shape of raw apple cells. Their method involves enzymatic treatment coupled with heat processing to separate the cells from the

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raw tissue. This double treatment can weaken the middle lamella, thereby affecting the size and shape of the cells as little as possible while increasing the rate of degradation of the middle lamella and therefore separation of the cells. Using image analysis software (ImageJ) and optical microscopy, they highlighted 5 different size distributions of cells for 5 varieties of apples. Nonetheless, the method they proposed yields only two-dimensional (2D) information and thus no information on cell volume.

Confocal scanning laser microscopy (CSLM) has the advantage of visualising tissues in 3D and allows investigators to examine the interior of a sample without being a destructive method. Using CSLM, Lapsley, Escher, and Hoehn (1992) have been able to access 3D information on internal structure and cell cohesiveness of intact apple fruits of various cultivars. In 1999, Gray, Kolesik, Høj, and Coombe (1999) reconstructed grape berry parenchymal cells in 3D by means of confocal microscopy. To obtain these images, they stained the cell wall with safranin O. 3D reconstruction enables analysis of cells as geometric solids and consequently measurement of the surface area and volume of cells. This work shows that cells are irregular in shape. Cell volume is reported to be between 40×10^3 and 400×10^3 μm^3 and cell shape variability has been highlighted by the S/V ratio from 0.08 to 0.198 μm^{-1}. On the other hand, these results have been achieved on raw parenchyma: cells thus remain all attached together via the middle lamella and have preserved their turgor. In fruit purees, cells have lost their turgor pressure and are more or less detached. It then becomes more difficult to directly examine stained samples by confocal microscopy, especially in purees with high cell wall content. It is therefore necessary to develop a method for examining plant cells in suspension in concentrated domains.

Because confocal microscopy provides some insight into the overall microstructure of food systems (Lopez-Sanchez et al., 2011), the literature also highlights the use of labelling and confocal microscopy to elucidate the relation between structure and properties of complex food matrices. Accordingly, by combining gluten labelling and confocal microscopy during mechanical treatment, Boitte, Hayert, and Michon (2013) were able to study the evolution of the gluten network's microstructure in the course of deformation of wheat flour doughs. Another example combining specific staining and confocal microscopy is the study by Huc, Moulin, Mariette, and Michon (2013) who showed that organisation of a fat and protein network has an impact on the mechanical properties and eye growth of Swiss-type cheeses.

In the present study, we describe a new method for assessing the volume of cooked parenchymal cells in concentrated media and for obtaining answers about the way apple cells compress when particle concentration increases. The purpose of our work is not to provide results for statistical analysis of a particle size distribution but actually to offer an innovative protocol for assessing compressible particles' volume in a highly concentrated suspension. This approach is of great interest for a better understanding of the rheology of compressible particles in general.

2. Materials and methods

2.1. Plant material and processing conditions

Results presented in this work were obtained on apple purees composed only of mature Golden Delicious apples (*Malus domestica* Borkh. cv Golden Delicious). The apples were turned into a puree by a French manufacturer (Conserves France) following an industrial hot break process.

2.2. Red Congo staining

Apple cell walls were stained with a 1% Congo Red (w/w) aqueous solution (Sigma–Aldrich).

2.3. Optical microscopy

Light microscopy was used to examine the overall structure of the samples in suspension. Two microliters of each sample was placed onto a glass slide and covered with a cover slip. Samples were examined with a 2× or 10× objective lens under an Olympus BX51 microscope in phase contrast.

2.4. Environmental scanning electron microscopy (ESEM)

ESEM (FEI Quanta 200) was employed to measure the thickness of apple cell walls. Samples were observed under the following conditions: secondary electron detector, ESEM mode (GBSED: gaseous BSE detector), pressure of 665 Pa, voltage of 5 kV, and a spot size of 4. A temperature of 2 °C was set to prevent solvent evaporation using a Peltier stage. Five measurements of the cell wall thickness were conducted on five different pictures.

2.5. Confocal microscopy

Confocal microscopy was performed by means of inverted microscope Leica TCS SP8. Samples were placed onto a glass cover slip using a spacer. A spacer 5 mm wide (500 µL) was specially designed with a 3D printer and was not entirely filled with the sample to avoid extra compression of the cells and to keep their original spatial arrangement. The spacer was covered with another cover slip to prevent solvent evaporation during image acquisition. The samples were examined via a 40× water objective (NA = 0.8). Fluorescence of Congo Red was detected using an Argon laser. The wavelength of absorption was set to 488 nm, and emission wavelength was between 498 and 650 nm. Image size was set to 512 × 512 pixels, which corresponds to 291 µm × 291 µm. Images were acquired every 0.802 µm in the z-direction.

Images of fluorescent microspheres were captured under the same conditions and were used to check the accuracy of volume and surface measurements in image analysis.

2.6. Image analysis

Image pre-processing and analysis were conducted in the Scan IP™ software, version 7.0, build 2656 (© 2000–2014 Simpleware Ltd.).

2.7. Statistical analysis

This analysis was carried out in the XLStat software, version MacOS X 2016 (© 2016 Addinsoft SARL, Paris, FRANCE). The results were expressed as mean ± standard deviation. Comparisons of means were made by the Newman–Keuls test at $p < 0.05$.

2.8. Rheological measurements

These measurements of model suspensions were conducted by means of a stress-controlled rheometer (MCR301, Anton Paar) equipped with large-gap coaxial cylinders (CC18.92; inner radius: 9.46 mm, outer radius: 14.46 mm, gap: 5.00 mm, gap length: 40.00 mm), according to the protocol described elsewhere (Leverrier, Almeida, Espinosa-Munoz, & Cuvelier, 2016). Apparent viscosity was quantified at 50 s^{-1}.

3. Description of the experimental strategy

3.1. Preparation of model suspensions

Observation of cells in concentrated suspensions was performed on model suspensions composed only of individual parenchymal cells. A separation protocol was thus set up to prepare single-cell suspensions. Processing conditions are summarised in Fig. 1.