Modelling of plant tissue microstructure for Finite Element Method

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ABSTRACT

This paper reviews three different methods for parameterisation of plant tissues which can be used to create models for the simulation of the mechanical behaviour of biological cellular structures. Vectorisation, Voronoi tessellation and ellipse tessellation were tested. Potato tuber and carrot parenchyma were chosen as examples. For each method tested, five geometrical parameters were analysed: area, perimeter, orientation, elongation and a local indicator of spatial association of all individual regions which represented cells. The reconstruction accuracy of the original tissue microstructure by each parameterisation method was investigated by the comparison of the geometrical properties of the cells from the segmentation with their virtual equivalents. Based on the results, Voronoi tessellation was considered to be inaccurate for tissue modelling. The vectorisation procedure only allowed for reproduction of the general shapes of cells, and the curvature of cell walls was neglected in this method. For both the Voronoi tessellation as well as vectorisation, created cells completely filled the space with no additional gaps and possessed sharp, angular shapes. The best overall reconstruction accuracy was obtained with ellipse tessellation. Models created with this method can be considered as representative equivalents of real tissues in terms of cell area, orientation, perimeter, shape and spatial arrangement.

Keywords: tissue modelling; tessellation; CSLM microscopy; image analysis

INTRODUCTION

Fruits and vegetables are important components of human diet. They contain vitamins, microelements and antioxidants, that protect us from many diseases. Today's consumers expect the highest quality of food products. Providing them in such a state is a great challenge for food producers, distributors and sellers. Texture is one of the most important attributes defining the quality of many fruits and vegetables. Consumers often prefer a crispy, crunchy and juicy products, because these characteristics are associated with freshness. An equally important factor affecting the quality of fruits and vegetables, which largely leads to a significant loss of their commercial value is the occurrence of the mechanical damages. Both, the texture understood as a sensual parameter, as well as the susceptibility to the damage are strongly dependent upon the plant tissue mechanical properties. Hence, there is a need to study and describe the process and consequences of the plant tissue deformation. Knowledge of the tissue deformation mechanisms could allow us for better prediction and minimization of the quality losses of the food products.

Mathematical models have a great importance in explaining the phenomena and structural features that underlie the observed mechanical properties. However each model is a form of idealization, and its prediction abilities are determined by the level of simplification of the structure and physical properties of the real system. Observations of plant tissues on the microscale reveals a complicated structure. These tissues are composed of large numbers of cells whose shape, size and distribution show high variability, not only between species or varieties, but even within individual plants [1,2]. Depending on the type, spatial arrangement, shape of the cells, amount of intercellular air space, turgour, nano-composition of cell walls or the degree of degradation of middle lamellae, tissues may exhibit different macroscopic properties responsible for postharvest quality [3,4,5,6].

One of the most promising methods, that allows us to simulate the behavior of systems of such complexity is the finite element method. First steps in solving a problem, before the appropriate analysis with FEM, is to create a physical model of the tested object. The physical model is defined by its geometry, material properties and boundary conditions such as loads and the type of supports. It is important to create a proper model with the highest possible degree of accuracy regarding real shape reconstruction; but on the other hand, the model must be simple enough to allow for efficient calculations.
Since it was proved that the confocal scanning laser microscope (CSLM) is a useful tool for obtaining images of plant tissue that would be relatively easily segmented, the goal of this research was to identify the method of parameterization which will be used for FEM, and on the other hand, will properly simulate the real structure of plant tissues. Three different methods were tested: vectorization, Voronoi tessellation and ellipse tessellation. The methods were tested in terms of the accuracy of the reconstruction of potato and carrot tissue microstructure in relation to the segmentation method on images obtained using CSLM, as previously demonstrated by Zdunek et al. [5].

MATERIALS & METHODS

In order to investigate the robustness of each of the parameterisation procedures, potato tuber and carrot parenchyma were chosen. These materials were previously investigated by Zdunek and Umeda [4,6] using CSLM and in the present paper, the same images were used. The images were previously segmented using a protocol developed by Zdunek et al. [5], and this method is considered here as the reference. The segmentation was performed using Aphelion v.3.2 (Adcis, France) image analysis software. As a result of segmentation, binary images of skeletons of the cellular structure were obtained.

For the each method tested, five geometrical parameters were analysed: area, perimeter, orientation, elongation and a local indicator of spatial association of all the individual regions which represent cells. To describe the spatial arrangement of geometrical parameters, the local variation of Moran’s I (LISA – the local indicator of spatial association) autocorrelation statistic was used which enabled us to measure the degree of similarity of an observation in relation to its neighbours and to show the statistical significance of this relationship [7,8].

The first proposed algorithm, called the vectorization, simplifies the shapes of cells through the transformation of the digital image of the thin skeleton to a set of vertices and edges where cells are represented by polygons. The protocol searches for pixels within the image which have more than two neighbours. Points with three or more neighbours, called junctions, indicate the intersection of adjacent cell walls, and they are used as the vertices of virtual cells. Since the points designated as the vertices of a polygons must be assigned to the appropriate objects, the protocol analyses each cell separately with the use of a contour trace algorithm. As a result of these operations, for each cell is assigned a set of points which, when connected, form a virtual cell.

Voronoi tessellation is the second of the proposed methods. It uses the centres of mass of the cells from microscopic images (Centroid – Based Voronoi Diagram algorithm, [9]) to generate Voronoi cells in 2D space, embedding those points and their domain of attraction. This domain is specified by a mathematical definition which says that for a given point \( p_i \), called the generator, the Voronoi cell \( V(p_i) \) is a part of the 2D plane containing all points \( x \) at least as close to the generator as to any other \( p_j \). The equation expressing this definition is as follows [10]:

\[
V(p_i) = \{ |p_i - x| \leq |p_j - x|, \forall j \neq i \}
\]  

All the virtual cells produced by this method were in shape of convex polygons. In contrast to the vectorisation method, the Voronoi tessellation algorithm allowed the creation of diagrams based on the statistical distributions of tissue morphological descriptors. This excludes the necessity of obtaining real tissue microscopic images for every created model.

The third method was developed on basis of the ideas presented by Mebatsion et al. [9]. The algorithm uses the method for direct least squares fitting of the ellipses to find the best fit ellipse for the boundary data obtained from each cell. For exact details of this solution, the reader should refer to the original publications [11,12,13]. The procedure developed in MATLAB starts from the fitting of ellipses to the shapes of real cells. When all cells have their virtual equivalents assigned, the algorithm determines the intersection points for each pair of neighbouring ellipses. Then straight lines are determined through pairs of intersection points. When neighbouring ellipses overlap, they are trimmed along constructed lines.

The reconstruction accuracy of the original tissue microstructure by each parameterisation method was investigated by comparison of the geometrical properties of the cells from the segmentation with their virtual equivalents. Linear regression models between the reference and modelled parameters were built. The reconstruction performance was expressed in terms of the coefficient of determination \( R^2 \), the slope of the regression line (which was expected to be close to 1) and the root mean square errors for prediction (RMSEP) of linear regression models between the reference and modelled parameters. Also, the RPD value (ratio of prediction to deviation) was calculated as the ratio of the standard deviation to the RMSEP. RPD evaluates...
the accuracy of a model by the ratio of the variability of observed values to the variability resulting from model mismatch. An RPD value below 1.5 indicates that the model is not useful and reconstruction of tissue microstructure is unsatisfactory. When the value for RPD is higher than 2.0, the obtained structure can be considered as a representative sample of the modelled object [14].

**RESULTS & DISCUSSION**

Voronoi tessellation was found to be the least accurate of the three methods used in this study. Area, perimeter, orientation and shape of the virtual cells differed widely from the measured values. This was confirmed by the regression coefficients of the models for potato and carrot. All values were much above or below 1, which meant that the parameters of the cells were over or underestimated by Voronoi tessellation.

The calculated RMSEP values also indicated the highest estimation errors for Voronoi tessellation in the case of each geometric parameter. Determination coefficients showed that only a small percent of the response variation could be explained by the regression model ($R^2$ from 0.64 for area to 0.09 for elongation). RPD values below 1.5 for geometric parameters further showed that Voronoi tessellation models cannot be used as a representative equivalent for carrot or potato tissue. The obtained results also proved the inability of this model to reconstruct tissue topology (the highest RMSEP values, with $R^2$ and regression coefficient values equal to 0.553 and 0.386 for potato and 0.711 and 0.490 for carrot respectively).

![Figure 2. Examples of created linear regression models between the reference and modelled parameters of carrot cells: a) segmentation vs. vectorization for area, b) segmentation vs. ellipse tessellation for perimeters, c) segmentation vs. vectorization for elongation, d) segmentation vs. ellipse tessellation for elongation.](image)

The vectorisation method allowed us to retain the original spatial arrangement of the tissue. This was indicated by the regression and determination coefficients equal to 0.98 and 0.87, respectively, with an RPD value of 3.23 for potato. Similar results were obtained for carrot tissue models (reg. coeff. of 1.06, $R^2$ of 0.94 and RPD of 4.5). The RPD value of 6.29 and $R^2$ value of 0.97 showed that the results for area can be considered as satisfactory (Fig. 2. a). Very poor predictions were obtained for the orientation of cells in this...
case. The created models had only about a 69% accuracy in the reconstruction of cell orientation. Modelled cells with vectorisation were more elongated than those from the microscopic images. The RPD for elongation was greater than 2, but the combination of low coefficients of determination ($R^2$ of 0.71) and regression (0.63 and 0.67 for potato and carrot, respectively) clearly showed that vectorisation also gave poor results in the case of elongation (Fig. 2. c). Vectorisation achieved the best results among all the methods for the perimeters of cells. The regression coefficients and $R^2$ close to 1, combined with the lowest RMSEP and the highest RPD value, confirmed this supposition.

The best representation of the real tissue for almost all parameters was obtained using ellipse tessellation. This method was superior in the reconstruction of area Examples of created linear regression models, orientation, elongation and spatial arrangement of real cells. It had an RPD value at least twice as high and a lower RMSEP for those parameters compared to vectorisation. These proportions were the same for the potato and carrot tissue models. The determination and regression coefficients were close to 1, with the only exception in the case of elongation (Fig. 2. d). Similar to vectorisation, virtual cells created with use of ellipse tessellation were more elongated than real cells, but this deviation was smaller than that observed with vectorisation.

Lower performance of this method was observed when determining the perimeters of cells. Calculated perimeters were smaller than those of real cells (reg. coeff. of 1.20 and 1.16 for carrot and potato respectively, Fig. 2. b). Linear regression for this parameter also had a higher RMSEP and regression coefficient than in the case of the vectorisation method, but even so, the accuracy of ellipse tessellation for these perimeters was high with an RPD value of 4.62 and an $R^2$ value of 0.96 for potato and an RPD value of 4.19 and an $R^2$ value of 0.97 for carrot.

CONCLUSION

The comparison of tissue models obtained by Voronoi tessellation, ellipse tessellation and developed vectorisation methods showed that Voronoi tessellation (CVD) was inaccurate for tissue modelling. This method had difficulties in the reconstruction of both isotropic potato parenchyma and heterogeneous carrot tissue. Cell walls of the virtual cells were not covered perfectly with their equivalents from the microscopic images. Virtual cells from Voronoi tessellation had geometrical parameters different from the original and also a different spatial layout.

The vectorisation method showed good results for the reconstruction of cells in terms of area and spatial distribution. This method also produced the results closest to the original perimeters of the virtual cells. Apparently, in the case of tissues with densely-packed cells such as observed in carrot or potato, using polygons as cells representations does not cause significant changes of their sizes. The problem that should be considered in the case of this method is oversimplification of cell geometry, since the curvature of the cell walls was neglected and their edges were sharp at the intersection of adjacent virtual cells. This may contribute to difficulties when models are applied to the finite element method. The representation of complex shapes with a higher level of detail would be more appropriate.

The best overall reconstruction accuracy was obtained by ellipse tessellation. Models created with this method can be considered as representative equivalents of real tissues in terms of cells area, orientation, perimeter, shape and spatial arrangement. However, because of the ability to create only convex shapes, elliptical tessellation would be not able to reconstruct large intercellular spaces for some other materials, like apple for example. However on the other side , rounded shapes of virtual cells produced by this method create small spaces between the corners of cells, which would exist actually in the real tissue and there are not visible on the confocal images of this scale.

REFERENCES


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