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Unravelling Interactions and Mechanical Properties of Plant Cell Wall Biopolymer Using TA.XT plus Texture Analyser †

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Abstract: The plant cell walls (PCW) act as a skeleton providing mechanical protection to the plant cell. Its composition and the interaction between its components partly determine the mechanical properties of the PCW. Understanding the contribution of cell wall components to cell walls properties is challenging, due to the heterogeneity of plant tissues and the ability of plants to adapt and modify cell wall composition in response to environmental stimuli. In this publication, interactions between individual PCW components have been analysed by used of composite macro-alcogels based on cellulose-xylan blends dissolved in ionic liquid 1-Ethyl-3-methylimidazolium and a texture analyser. The results showed a reduction of the young modulus of the alcogels upon addition of xylan to cellulose mixtures. Additionally, Voigt and Reuss theoretical models suggest that at proportions of 20:80 and 50:50 xylan-cellulose, the polymers could interact modulating the mechanical properties of the mixtures. In conclusion, the analysis of cell wall component' mixtures using a Texture analyser offers an innovative approach for the screening of agricultural waste available for exploitation in new materials.

Keywords: plant cell wall; mechanical texting; polymer interactions; young modulus

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1. Introduction

The specific composition and interactions between its components define the mechanical properties of the PCW, which is important for providing protection, support, and integrity to the plant cell. During the development, the cell wall mechanics should reach a balance between flexibility and integrity to protect the plant as well as to allow that different processes, such as cell expansion for growth, occur [1]. The study of the mechanical properties of the cell wall is of crucial importance to understand different biological processes of the plant. Its understanding is also relevant to other disciplines such as the food [2] and paper [1] industry, medical engineering to develop scaffolds [3] drug delivery systems [4].

The study of the contribution of certain cell wall components to the mechanical properties of cell walls is complicated, due to the heterogeneity of plant tissues/organs and the ability of plants to adapt and modify cell wall composition in response to environmental stimuli [5]. Different approaches have been developed to study the mechanics of the cell wall or the interactions between polysaccharides. Most of these methods involve measuring material deformation in response to the application of a known force, including, for example, nanoindentation coupled with Atomic Force Microscopy [3,6,7] or other micro-indentation systems [8]. More recently, a system was developed using the scattering of light interacting with the acoustic waves (¨Brillouin scattering¨)for non-invasive measurement of cell wall mechanical parameters [9]. Also, in recent years, an automated confocal micro-extensometer (ACME) was designed to track cell wall mechanics in vivo. ACME allows measurement of reversible (elastic) or irreversible (creep) deformation at the organ or individual cell level [10]. These methods are difficult to set-up, rely in highly qualified expertise and expensive equipment which can limit their routine application in regular labs. There are also numerous factors that can affect the reproducibility and accuracy of the measurements or make their interpretation difficult, e.g., the conditions in which cell walls are kept and the experimental environment, the cell and tissue topography, curvature, and other geometrical features.

The texture analyser or tetrameter is an instrument used to measure mechanical properties of materials. The main principle of the technique is based on the measurement of the material's deformation, which is produced when a controlled force is applied (Texture Analyser, product specification). Texture analyser has been used to study the role of individual biopolymers on the properties of PCW [11,12].

In this publication a method is presented to analyse the properties and interactions of PCW individual components by mimicking the cell wall tri-dimensional matrix using a simplified model of composite macro-hydrogels & alcogels and a texture analyser. Hydrogels have been defined as a two or multicomponent system consisting of three-dimensional network of polymer chains and water that fills the space between macromolecules resembling cell walls [12]. To make hydrogels, glycan polymers are solubilized, and hydrogen bonds reconstituted in water to form a network. We use the ionic liquid 1-Ethyl-3-methylimidazolium acetate (Emim Ac) as an effective solvent for a variety of cell wall glycans, including cellulose, without inducing misleading modifications in the polymer structure [13]. Ionic liquid solutions are coagulated in water or methanol leading to the formation of macro 3D network structure mimicking the cell wall net. Mechanical properties of the hydrogels are tested using a penetration test in compression mode with a Texture Analyser. As a result, a force-displacement curve is obtained and several mechanical parameters, such as elasticity, stiffness and brittleness, can be calculated. Their study will also reveal polymer interactions that exist and modify cell walls mechanical properties thus plant growth and development. As an example, hydrogels made of mixtures of cellulose and xylan are analysed. The results confirm the applicability of this method in the screening of interactions between cell walls polymers.

2. Experiments

2.1. Material

Highly purified xylan from beechwood was obtained from Megazyme (https://www.megazyme.com/). Microcrystalline cellulose (Avicel), the ionic liquid 1-ethyl-3-methyl-imidazolium acetate ([C2mim] [OAc]) (95% purity), methanol (≥99.9%) and ethanol (absolute, ≥99.8%) %) were obtained from Sigma Aldrich.

2.2. Hydrogels Preparation

Alcogels were prepared following the protocol described in Abou-Saleh et al**.** (2018) [14]. Xylan and cellulose were ground using a TissueLyser (Qiagen, Hilden, Germany) and dried at 55 °C for at least two days before the sample preparation. The solutions were prepared in an MBraun Labmaster 130 atmospheric chamber under nitrogen, providing a dry environment, with the chamber being maintained at a dew point level between −70 and −40 °C. Five different cellulose blends were prepared with concentrations of 0, 20, 50, 80 and 100% xylan all at a final concentration of 10% (w/w) of total carbohydrate weight in the ionic liquid 1-Ethyl-3-methylimidazolium (Emim Ac). After dissolution, usually after 7 days at 55° Celsius with continuous agitation, the Emim Ac was eliminated by repeated washing with methanol to re-precipitate the polysaccharides and obtain a gel like solid structure.

2.3. Analysis of Mechanical Properties by Texture Analyser

The texture analyser TA.XT plus (Stable Micro System Ltd., Surrey, UK) equipped with a flat-bottomed probe of 2.0 mm diameter was used to determine the properties of the hydrogels by applying indentation in compression mode. TA.XT software (Exponential) contains a library with predefined tests. The 'return to start' test was selected for this specific purpose. In this test, the force needed to indent the hydrogel's surface is measured.

Data of force (N) and distance (mm) was recorded and graphed in "Force-distance curve". The force-distance curve resulting from the test was used to identify and calculate texture parameters (Figure 1A). Two points, fracture point and maximum displacement, can be distinguished. The fracture point is the force necessary to cause the first fracture on the hydrogels. Maximum displacement corresponds with the value of the displacement where the first fracture is produced [15]. Additionally, young's modulus or elastic modulus can be calculated from the curve. The Elastic modulus or Young's modulus (YM) was obtained using the following equation (Flat punch model):

$$YM = \frac{F\,(1-\upsilon^2)}{2R\delta}$$

where E is the elastic modulus (or young's modulus) (MPa), F is the applied force (N), δ is the displacement of the elastic region (mm), R is the probe radius (mm) and ν is Poisson's ratio of the specimen. $\nu = 0.35$, described previously, for hydrogels [14].

2.4. Analysis of Polymer Interactions in Xylan-Cellulose Blends

Experimental data was compared with a mathematical model applied to determine the ideal or theoretical stiffness values of two materials in a blend[16]. Voigt and Reuss theoretical models describe the mechanical response of two idealised combinations of materials. Voigt model increase to the greatest possible value of the stiffness by having the stiffer component expanding the sample in the direction of strain and the Reuss model minimise the stiffness by having the stiffer component in layers orthogonal to the strain direction [14]. Deviation from these bonds suggests a change at molecular level that can be translated in an interaction between molecules. Equations to calculate the Voight and Reuss upper and lower limit of stiffness are:

$$YM\ Voigt = YM1 * V1 + YM2 * V2$$

$$YM\ Reus = \frac{YM1 * YM2}{YM1 * V2 + YM2 * V1}$$

where YM is the Young's or elastic modulus (MPa) and V is the volumen or weight of the components in parts per one: $V1 + V2 = 1$.

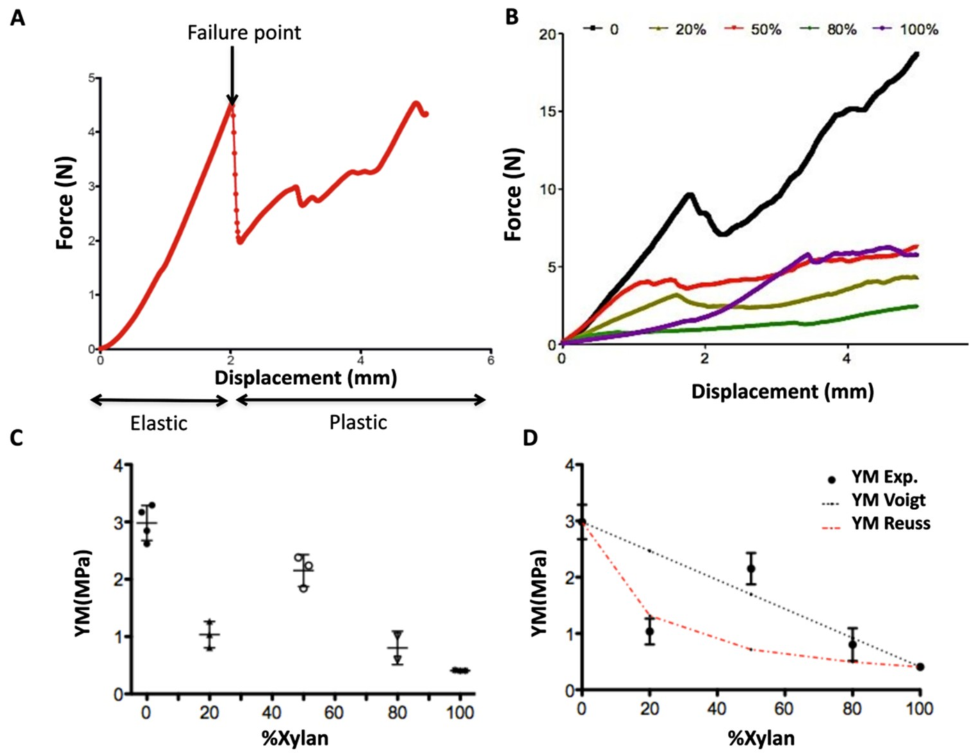

Figure 1. Mechanical properties of xylan-cellulose hydrogels. Force-Displacement curve for a glycan hydrogel (**A**). The graph represents force (in N) on the vertical axis and displacement or depth (mm) on the horizontal axis. Different regions of this curve after a penetration-compression test are shown. The linear part of the curve represents the elastic behaviour of the hydrogels, until the Failure Point that indicates the limit of the elastic behaviour region and the beginning of the plastic behaviour region. Force-displacement curve (**B**) of xylan-cellulose hydrogels coagulated with methanol at different xylan concentration (0% in black, 20% in green olive, 50% in red, 80% in green and 100% in magenta) using a Texture Analyser in compression mode. Young's modulus (**C**,**D**) of individual data correspond to four-five independent replicas the whiskers represent the media with standard deviation (SD). Representation of Voight and Reuss theorical models describe the mechanical response of two idealised combinations of materials (**D**).

3. Results

3.1. The Young Modulus Is Reduced upon Addition of Xylan to Cellulose Mixtures

To study cellulose-xylan properties, alcogels were produced with varying amounts of Avicel and beechwood xylan. Pure cellulose is labelled 0% (no xylan in the mixture), 20:80 is 20% xylan and 80% cellulose, 50:50 is 50% xylan and 50% cellulose, 80:20 is 80% of xylan and 20% cellulose and 100 is 100% xylan without cellulose. Figure 1B shows an example of force-displacement curve obtained when xylan-cellulose alcogels are probed in a texture analyser as described in the methods. From these curves, the YM and hydrogel toughness or hardness can be measured using the 'Flat punch model' to derive YM from the linear elastic region and the maximum displacement point (displacement where gel failure occurs) as an estimate of hydrogel toughness. Analysis of the linear elastic region revealed that the YM of the blend decrease from 3.4 MPa in 100% cellulose alcogels to 0.4 MPa in 100% Xylan alcogels (Figure 1C). Intermedial concentration values do not follow a linear trend suggesting potential interactions between these glycans.

3.2. Young Modulus Value at Different Xylan Concentrations Are Outside the Voigt-Reuss Predictions

According to the "ideal mixing law", the properties of two components in a mixture are found to be the result of the proportional combination of each component. Plotting elastic modules (YM) vs. proportion of the component from 0 to 100% a linear dependence should be found in case of ideal mixture. Deviation from this linear dependence can indicate the formation of additional compounds, aggregates hydrogen bonds, micelles or the dissociation of and associated liquid [17]. Voigt and Reuss theoretical models, which are developed to predict the mechanical properties of elastic composite materials, were used to inspect the possible interactions between these polymers. Figure 1D shows the values of the Young's modulus with respect the xylan concentration and the data obtained from calculating the upper and lower boundaries with Voigt and Reuss theorical models. Differences between the theorical and experimental value were calculated. Slight deviations (around 20%) from the theoretical model were found in a 20% xylan-cellulose blend and 50% xylan-cellulose. This could indicate that the mechanical properties of both glycans at this specific concentration in the alcogel are not only due to their individual properties and interaction at molecular level can be affecting the ideal behaviour of these molecules.

4. Conclusions

In conclusion, hydrogel technology and the use of a Texture Analyser was combined to analyse mechanical properties and interactions of the cell wall glycans xylan and cellulose. Alcogels made of mixtures of commercial cellulose Avicel and xylan from beechwood were analysed with a Texture Analyser and the results are reported here for first time. Texture measures of cellulose-xylan blends suggested that different concentration of the xylan can modulate the mechanical properties of the cellulose reducing the rigidity and increasing the elasticity of the blends. The YM values does not follow a linear trend suggesting interactions between these glycans depending on the concentration. Voigt and Reuss theoretical models suggest that at proportions of 20:80 and 50:50 xylan-cellulose the polymers could interact modulating the mechanical properties of the mixtures. The nature, veracity and effect of these interactions in the cell wall context is yet to be verified. In support, previous research showed that reduced xylan content weaken primary cell walls and cellulose- xylan interactions were detected by solid state Nuclear Magnetic Resonance [18]. Although at different scales, our results using alcogel polymer mixtures and a Texture analyser, align well with xylan-cellulose interactions microscopically observed using NMR. Therefore, we propose the applicability of our simplified method in the quick screening of interactions between cell wall polymers. By applying this method to the study of plant derived materials, new applications can be foreseen such as the screening of agricultural waste for exploitation as sustainable materials.

Author Contributions: C.P. did the experiments, data analysis, interpretation of results and writing of this manuscript. Y.B.-A. supervised the experiments and the writing of the manuscript.

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Abbreviations

The following abbreviations are used in this manuscript:

PCW	plant cell wall
YM	Young's modulus
NMR	Nuclear Magnetic Resonance.
MPa	megapascal (MPa = 10^6 Pa)
N	Newton
mm	millimeter

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