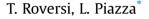
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Supramolecular assemblies from plant cell polysaccharides: Selfhealing and aging behavior



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ABSTRACT

A fundamental understanding of plant cell wall rheology is essential in view of the formulation engineering of plant-based foods/ingredients. In this paper, by means of oscillatory measurements, we study the rheological behavior of water suspensions of cell wall polysaccharidic supramolecular assemblies, which are extracted with selective solvents precipitation method from apple flesh. Once rehydrated with water, they behave as gel like materials, as it results from the mechanical spectra, with the elastic modulus *G'* higher than *G"* within the frequency range considered. Interestingly, amplitude and time sweep measurements show reversible stress softening and stress recovery after high shear strain. The observed behavior of the cell wall molecular assemblies resembles closely to that of colloidal gels, which also display shear-thinning rheological properties and a complex aging response. In this regard, creep measurements indicate history-dependent effects, which are interpreted in terms of aging phenomena, similar to those existing in glassy systems.

The self-healing behavior and the non-equilibrium nature we observed allow customized modifications during processing and storage to obtain new structural states and rheology. Such changes in the microstructure might be relevant for product and process performance.

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1. Introduction

To perform its key role in growth, cell differentiation, intercellular communication, water movement and defence, the plant cell need an extraordinary capacity to regulate its shape and mechanics. This capacity stems from the unusual physical properties of a complex three-dimensional polymers network, i.e. the cell wall.

The cell wall is typically a thin, flexible layer that surrounds plant cells; it consists primarily of crystalline cellulose macrofibrils, providing the structural girders, that are linked to and surrounded by hemicelluloses and pectins (Cosgrove, 2005; Somerville et al., 2004). A small amount of structural proteins is also present (Cosgrove, 2000).

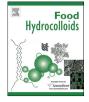
Besides providing support for cell expansion and plant growth, cell walls might play a key role in the field of biomaterials (Foster, 2011), and understanding the connection between microstructure and their mechanical properties is of primary importance among scientific community.

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In the recent years, consistent progress has been made in the study of the mechanical properties of solutions and network of plant cell walls polysaccharidic fraction. Reversible stress softening and stress recovery of cellulose fibers, which might play an important role during plant cell expansive growth, has been reported by Torres and co-workers in 2009 (Torres, Troncoso, Lopez, Grande, & Gomez, 2009). The micro-scale property commonly credited to be at the roots of this peculiar mechanical behavior is due to reversible nature of the process of fibers buckling with load, similar to what happens in dendritic growing actin filament (Chaudhuri, Parekh, & Fletcher, 2007). More recently, Vincent et al. (Vincent, Mansel, Kramer, Kroy, & Williams, 2013) revealed striking analogies between the rheological behavior of acid pectin gels with F-actin and collagen networks. The aforementioned experimental evidences suggest a complex interplay of pectins and cellulose macrofibrils in determining the overall mechanical properties of plant cell wall (Cybulska, Zdunek, & Kozioł, 2015). However, due to inherent structural complexity of plant cell walls, the mechanics of the whole structures are difficult to be ascribed to one single component. Their properties also depend on the manner in which the polysaccharides are interlinked to form the three-dimensional, functional structure of the intact cell wall (Jarvis, 2011).







Understanding the mechanics of this supramolecular organization is essential for new technological application, and it is of primary importance for designing biomimetic systems close to Nature's perfection with interesting and unexplored properties (Hamley, 2011). Parallel, in order to obtain critical information that can be used for product and process performance in the food industry, a fundamental understanding and control of plant cell-wall flow properties is required (Moelants et al., 2014). As a matter of fact, the rheology of these systems is considered a functional property because of its importance during the entire production chain up to the moment of consumption (Appelqvist, Cochet-Broch, Poelman, & Day, 2015) and digestion (Kong & Singh, 2008).

In the recent past, several studies have investigated the rheological behavior of water dispersions of cell wall materials (CWM) (Day, Xu, Øiseth, Hemar, & Lundin, 2010; Hemar, Lebreton, Xu, & Day, 2011; Herbert Kunzek, Opel, & Senge, 1997; Pickardt, Dongowski, & Kunzek, 2004; Vetter & Kunzek, 2003), which contains soluble as well as insoluble fiber in a partially retained cellular architecture. They have demonstrated dominant elastic properties that are dependent on the concentration, the stiffness and the elastic properties of the solid phase, which is largely controlled by the interactions between the solid particles and deformability of the full-packed particles. Yet, several peculiar features of the rheology of these systems remain poorly understood.

In this paper, through a detailed rheological investigation performed by oscillatory measurements, we discuss several features of the yielding and the restructuring processes in concentrated CWM water dispersions. In particular, amplitude sweep measurements display complete reversible stress softening of plant cell wall suspensions, and a peculiar two-stage recovery of the elastic behavior is highlighted by time sweep measurements. Finally, creep measurements show a spectacular long time response that proceeds in the opposite direction to the applied stress. The response becomes slower as the waiting time after shear melting increases, indicating history-dependent effects that are interpreted in terms of aging phenomena.

Our results therefore suggest concentrated cell wall dispersions to behave similar to soft glassy systems. This observation may have important implications for manufacturing and processing of cell wall material in industrial applications.

2. Materials and methods

2.1. Materials

Golden apples were bought from a local market in Milano, Italy. Methanol, chloroform, ethanol and acetone were of analytical grade and purchased from Sigma Aldrich. All the chemicals were used as received, without further purification.

2.2. Cell wall extraction

Cell wall materials (CWM) were extracted from apple flesh as previously reported (Campbell, Huysamer, Stotz, Greve, & Labavitch, 1990). Briefly, 20 g of fruit tissue were boiled in 95% (v/ v) ethanol for 20 min to inactivate potential wall-modifying enzymes, homogenized for 1 min and filtrated on glass microfibers filters, with a pore size of 125 μ m. A solution of Methanol-Chloroform 1:1 was used to wash the resulting pellet until complete discoloration. The pellet was washed in acetone to remove loosely associated water from cell wall material and finally dried at room temperature. After dehydration the obtained powder was milled by a rotor mill (Cross Beater Mill, Retsch GmbH, Germany) and finally sieved on an analytical sieve shaker (Octagon Digital, Endecotts Ltd., England), equipped with certified sieves. Before

rheological measurements, the obtained CWM were rehydrated with deionized water at 23 $^{\circ}\text{C}$ for 2 h.

2.3. Microscopy

Samples were examined with a BX43 light microscope (Olympus, Japan). Pictures were acquired with a CCD color camera XC30 (Olympus, Japan). Images were processed with a Digital Imaging Software cellSens Dimension Version 1.7 (Olympus, Japan). Dried CWM samples were mounted on glass slides and directly analyzed.

2.4. Rheological measurements

The rheological behavior of cell wall suspensions was characterized using a CMT (combined motor and transducer) rheometer (DHR-2, TA Instruments). All measurements were performed using stainless steel parallel plate geometry (40 mm diameter, gap 1.2 mm) at 23 °C. The influence of gap on the accuracy of rheological measurements was preliminary tested, and no significant differences were found in the range 0.8–1.5 mm. A solvent trap provided by the manufacturer for liquid samples was used to prevent loss of solvent.

First, oscillatory strain sweeps were performed to determine the linear viscoelastic region for each sample. To investigate the reversible stress softening of CWM dispersions, the first step of the test was performed with an oscillatory strain increase form 0.02–100%, and the second step was performed by decreasing the applied strain from 100 to 0.02% on the same sample. Frequency sweep experiments were then performed at low amplitude of strain (in linear regime) in the frequency range of 0.01–200 rad/s.

To investigate the recovery kinetics of CWM suspension after network destruction, viscoelastic properties of gel-like samples were measured as a function of time in an oscillatory time sweep (5 min, 0.3% strain, 1 rad/s frequency) before and after severe destruction of the gel network (100% strain, 1 min, 1 rad/s frequency). In order to determine the characteristic activation energy, creep test were also performed at various temperatures, in the range 30–45 °C.

The aging behavior of the suspensions was characterized by adopting a rheological method, as described by Coitre et al. (Cloitre, Borrega, & Leibler, 2000) for determining the creep responses of microgel pastes. At first, samples are shear melted¹ by applying a stress $\sigma = 150$ Pa larger than the yield stress for 60 s. This step is designed to erase all internal stresses coming from the loading of the rheometer and to prepare the gel-like suspension in a reproducible state. Secondly, at t = 0, the stress is set to zero and the samples are allowed to rest under zero stress for a time t_w . Finally, at $t = t_W$, a step stress $\sigma = 4$ Pa lower than the yield stress is applied to probe the mechanical properties of the samples.

3. Results and discussion

3.1. Soft-jamming transition

Cell wall materials extracted according to the protocol described in the experimental section contain soluble as well as insoluble

¹ Here and below, we borrow the idea of "*shear melting*" from the colloidal glass scientific community. It refers to the ability of soft-solids from colloidal glasses to be fluidized through shear. In colloidal glasses, such shear induced fluidization is one means of driving a solid-to-fluid transition within the generic jamming "phase" diagram, wherein strong similarities among shear-induced, concentration-induced, and temperature-induced fluidization exist. For details, please see (Eisenmann, Kim, Mattsson, & Weitz, 2010).

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