CHEMICAL ENGINEERING TRANSACTIONS

VOL. 43, 2015

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet

Chief Editors: Sauro Pierucci, Jiří J. Klemeš

ISBN 978-88-95608-34-1; **ISSN** 2283-9216

DOI: 10.3303/CET1543013

Self-Healing Cell Wall Particles Gels: a Rheological Investigation

Tommaso Roversi, Mirko Radaelli, Laura Piazza*

Dept. of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, Via Mangiagalli 25, 20133, Milano, Italy
laura.piazza@unimi.it

Plant and fruit cells encase themselves within a complex polysaccharide wall which are complex and highly sophisticated composite material made of cellulose, hemicellulose and pectin and which form a scaffold matrix with intertwined structure. In this work the viscoelastic properties of cell wall particles dispersions, which were extracted from fresh and aged apple flesh with selective solvents precipitation method, or alternatively with water as a solvent, were studied by means of steady and oscillatory measurements. The solid-like dispersions showed a shear thinning behaviour, and immediately recovered back into their original elastic character upon removal of the shear strain. This means that the cell wall particle dispersions have self-healing capabilities. They can be easily processed as low viscosity materials and subsequently form an elastic gel. Interestingly, the nonlinear mechanical response characterized by single step stress relaxation experiments, and re-plotted as isochrones of stress vs strain, revealed an early nonlinearity of the response and subsequent yield, as well as a clear time-dependent behavior. This behavior of the cell wall dispersions resembles closely that of colloidal gels, which also display shear-thinning rheological behavior and interesting aging response. We are therefore facing some structural properties, which can be of interest for new additives/ingredients to be used in food formulation technology. Cell wall materials, or some selected constitutive components, might hence play a key role as adaptable thickening agent in food formulations, due to their well-known ability to bind a large amount of water.

1. Introduction

A main challenge of food industry is how to ensure that basic consumers' needs for health and well-being are met for optimal throughout sustainable sources of materials and throughout reliable resource and energy efficient biotechnology-based processes. Along with this predominant strategy, there is a technical challenge for scientists to understand and manipulate the functionality of complex natural materials, which are available from plant and animal sources, in order to provide nutritious and good tasting offerings with functional health benefits. Often, natural complex molecular assemblies of biopolymers, extracted by means of easily scalable processes, are as yet relatively unexploited commercially. Results presented in this paper deal with biopolymers assemblies that were obtained by water and/or solvent extraction from apple flesh. Cell walls constitute the key structural components of fruit tissue. Since long time it is widely recognized that they play a central role in determining the texture quality characteristics of many fruit-based foods (Waldron et al. 2003). Apple tissue cells encase themselves within a complex polysaccharide wall, which constitutes the raw material that can be used to manufacture a number of products, from food to textiles. From a molecular point of view, these cell walls are complex and highly sophisticated composite materials made of cellulose, hemicellulose and pectin, which form a scaffold matrix with intertwined structure (Harris and Smith, 2006). There is an implicit assumption that by understanding the biophysics and molecular biology underpinning the physiology of cell walls materials (CWM), it should be possible to manipulate fruit tissue structure and therefore cell-wall-dependent quality characteristics. Equally, CWM added to foods are regarded as swelling and thickening agent, due to their ability to bind a large amount of water or as dietary fiber, so allowing innovative "functional

Please cite this article as: Roversi T., Radaelli M., Piazza L., 2015, Self-healing cell wall particles gels: a rheological investigation, Chemical Engineering Transactions, 43, 73-78 DOI: 10.3303/CET1543013

food" products with improved material and/or physiological properties (Kunzek et al. 2002). The technological functional properties of CWM are considerably modified during the extraction/preparation procedures, during the incorporation into foods (addition of water soluble components, stepwise reconstruction of the real food system) and generally during processing. The process-dependent external stress and the influence of internal conditions induce changes of the physicochemical properties of the food constituents, which consequently affect or determine the technological functionality. Crushed fruits, as well as their corresponding pureed and mashed products, are complex disperse systems. The dimensions from biopolymers to products span almost eight decades. For structure-building phenomena, the relevant length scale is mesoscopic, i.e. of the order of microns (Ubbink and Kruger, 2006). At the mesoscale some molecules assemble, as a first step in the formation of food structures. At this level, the main paradigms of a soft matter approach apply, according to which the governing physics is influenced by externally applied fields, such as mechanical stress. In this work the structural and viscoelastic properties of CWM aqueous dispersions, which were extracted with selective solvents precipitation method from apple flesh, are studied at the meso-scale structural level by means of steady and oscillatory measurements to evaluate their capabilities to be processed as low viscosity materials, in view of food formulation perspectives, or in view of forthcoming bioplastics.

2. Materials and Methods

2.1 Materials

Golden Delicious cultivar apples were obtained by a local market. Ethanol, methanol, chloroform and pure acetone were of analytical grade and purchased from Sigma Aldrich. All reagents were used as received, unless otherwise specified.

2.2 Preparation of cell wall materials and crude plant tissue dispersions

Cell wall material (CWM) and crude apple tissue (CAT) were both obtained from the apple flesh. For the extraction of cell wall materials, the fruit tissue was boiled in ethanol, homogenized and filtrated on glass microfibers filters with a pore size of 125 µm. The pellet was then washed in a methanol-chloroform solution, acetone and finally dried at room temperature.

For the preparation of the crude apple tissue, apple flesh pieces were heated in aqueous system, cooled at room temperature, homogenized and filtrated on glass microfibers filters with a pore size of 125 µm. Water was carried out by ethanol exchange and the pellet was finally dried at room temperature.

After dehydration, both the cell wall materials and the crude tissue were milled and sieved. In view of the rheological measurements, the powders were rehydrated with deionized water (final concentration: 4.7% w/w).

2.3 Rheological measurements

The rheological behavior of the dispersions 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) at 23°C. A solvent trap provided by the manufacturer for liquid samples was used to prevent loss of solvent. First, oscillatory stress 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 from 0.02 to 100 %, and the second step was performed by decreasing the applied stress from 100 to 0.02 % on the same sample. The nonlinear viscoelastic response of CWM dispersions was explored according to McKenna et al. (2009) for the stress−strain and stress relaxation responses assessments. Briefly, single-step stress relaxation experiments were performed for approximately 80 s with increasing deformation magnitudes. The just prior strain history was diminished by considering 800 s waiting time before measurement at the next larger deformation. This sequence was continued up to the maximum strain of 20%. The single step stress relaxation data were plotted at specific strain levels on modulus vs time coordinates, as well as on stress vs strain isochrones.

3. Results and discussion

3.1 Rheology of cell wall particles dispersions

The microstructure of the hydrated dispersions can be probed via viscoelastic measurements. In this paper, the linear viscoelastic properties of the apple CWM suspensions were first characterized via shear oscillatory measurements in the strain sweep mode. Furthermore, nonlinear mechanical response was investigated by stress relaxation tests, in which a precise deformation was applied and the stress developed was measured as a function of time. All the experiments were performed at a weight solid concentration of 4.7%. At this concentration, two distinctive features characterize the rheological behaviour of CWM dispersions (data not shown), namely:

1) the rheological complex modulus G* becomes independent by further increasing the concentration;
2) very long relaxation time develop, as measured by frequency sweep test.

These two findings suggest that at the investigated concentration, cell wall particles are jammed together and quenched in a glass-like structure.

Figure 1 shows the results of strain sweep test performed at low frequency (i.e. 1 rad/s) for 4.7 % w/w cell wall material dispersion in water. In the linear viscoelastic region, *G'>G"* and both moduli are independent on the applied strain. The material behaves as a solid and does not flow. Then, a stress softening region starts at $\gamma \approx$ 1 %, followed by a depletion of *G'* for almost three orders of magnitude at γ = 100 %. These results indicate cell wall particle dispersions to behave as elastic solid with an apparent yield. Surprisingly, during the second part of the test a complete reversibility of this stress softening was detected, as proved by the fact that dynamic moduli attained the starting absolute value. It can be inferred that hydrated cell wall material form gels by physical crosslinks which are not permanent, but can be destroyed and formed again very quickly. From a practical point of view, it can be concluded that CWM materials have self healing capabilities and can easily be processed as low viscosity materials, for example in intense flow processing, and subsequently form an elastic gel. The observed rheological behaviour can be seen as a high value property for textural food ingredients applications. From a material point of view, the behaviour discussed here resembles closely that of colloidal glasses, that is, networks consisting of percolated colloidal particles, which also display shear-thinning rheological behavior and interesting aging response.

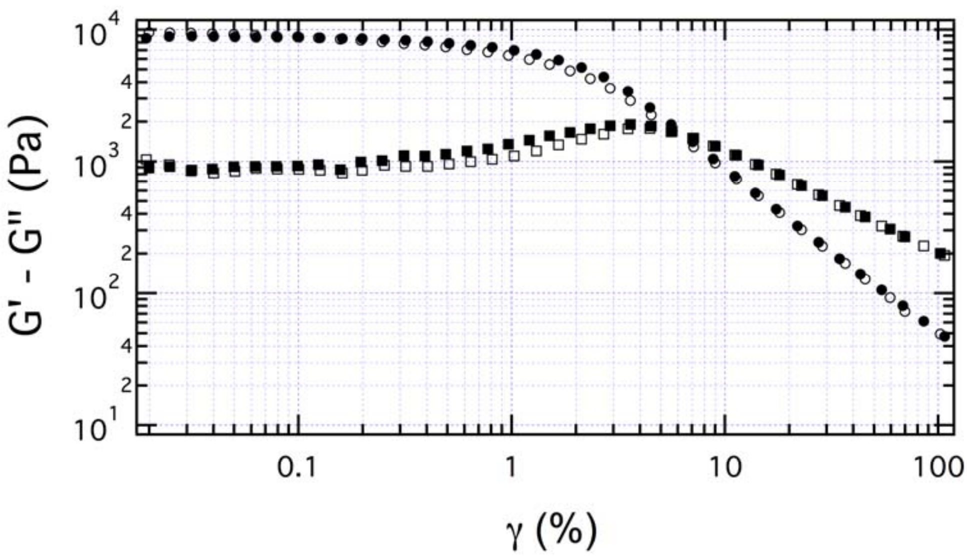

Figure 1: Strain dependence of the storage modulus (G', circles) and loss modulus (G", squares) of water dispersion of cell wall materials at 4.7% weight concentration. The test shows the existence of reversible stress softening (filled symbol and empty simple are the first and the second part of the test)

To better investigate the latter aspect, the relaxation response of CWM gels was investigated using the increasing step strain method described in the experimental part and adapted from McKenna et al (2009). Results are shown in Figure 2. The modulus at short times is of the order of 10,000 Pa and, as a general trend it decreases apparently with increasing strain.

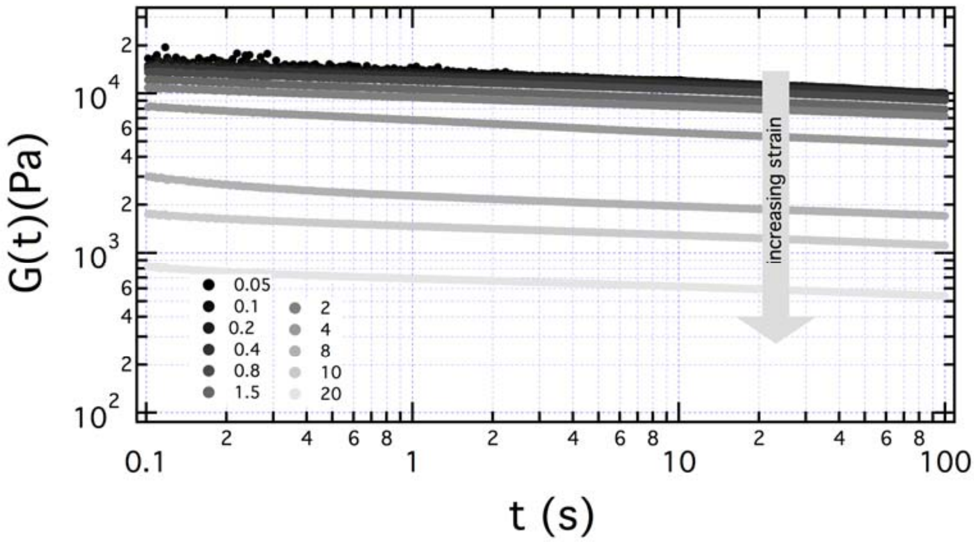

Figure 2: single step stress relaxation data for the 4.7% w/w cell wall particle dispersion in water. Different curves were obtained at different strains, as indicated in the legend

An alternative way to view the single step relaxation data is the isochronal plot, in a double logarithmic coordinates, of the stress against the strain (Figure 3). As stated by McKenna et al. (2009), this representation allows bringing out the apparent yield response, as well as the early nonlinearity of the response, which can be read as deviation from nominal slope of unity in the logarithmic representation. The hydrated cell wall materials exhibit a nearly constant modulus to a strain of around 1 %, after which the modulus starts to decrease. The yield stresses at different times are calculated by evaluating the flexion point in the logσ versus logγ curves, and shown in the inset of Figure 3. Interestingly, the stress−strain data cannot be superimposed on a master curve when the stress axis is normalized by the yield stress (Figure 4). Time dependence of the stress-strain data, which has been observed for polymeric or molecular glasses (McKenna et al., 2009), confirms the rheological similarity between cell wall particle dispersions and glassy liquids. The time dependent behaviour of cell wall particles gels are still under study.

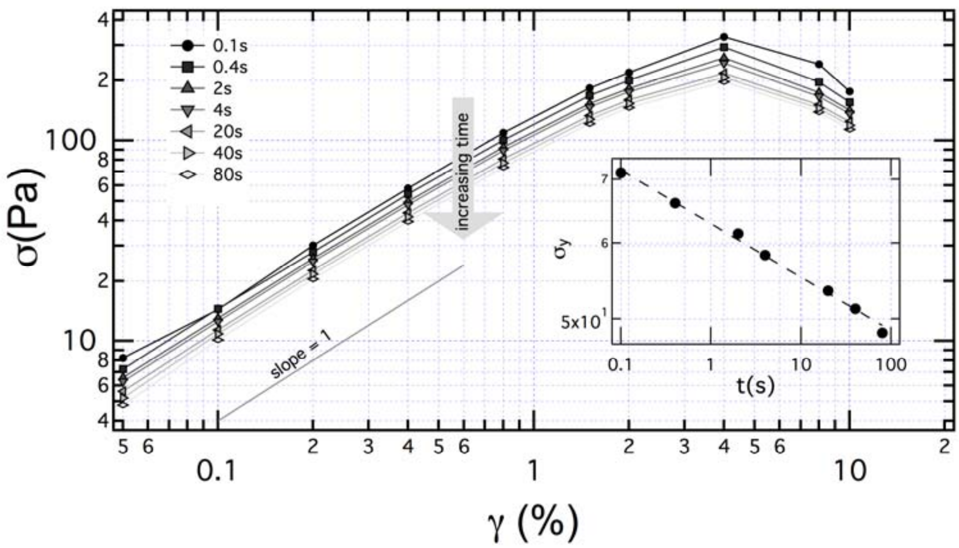

Figure 3: Double logarithmic representation of the shear stress-shear strain of the 4.7% cell wall particles dispersion extracted from stress relaxation data. Continuous line depicts slope 1 regime. Inset: yield stress values corresponding to each time

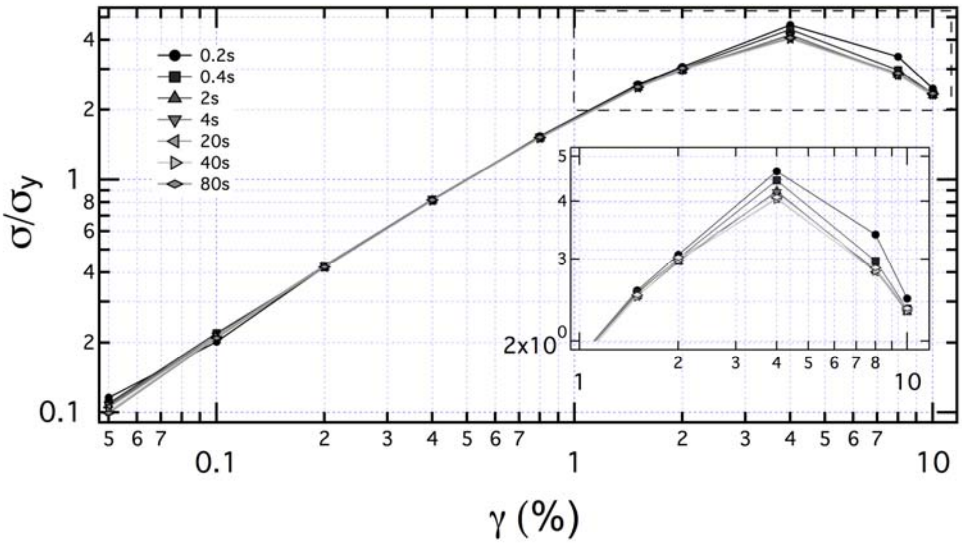

Figure 4: (main body) Normalization of shear stress data by yield stress (σ_y). (inset) Magnification of the 1%<γ<10% region, showing the non superposition of the stress-strain data

In view of designing specific functionality in specific food formulations, the non-equilibrium nature of these natural assemblies seems to enable some structural modification, to be accomplished during processing, aimed to obtain new structural states and rheology.

3.2 Rheology of crude plant tissue dispersions

With the goal of facilitating the scale-up of the extraction process above presented, a simplified extraction mode has been experienced and compared to the previous by assessing the rheological behaviour of the extracted components. A comparison was made between Cell Wall Material (CWM) water dispersions and Crude Apple Tissue (CAT) dispersions, at the same concentration. CWM is mainly composed of the alcohol insoluble substances, whereas the soluble fiber is nearly completely removed in the crude tissue. The CAT dispersions underwent a stress sweep test and results are shown in Figure 5.

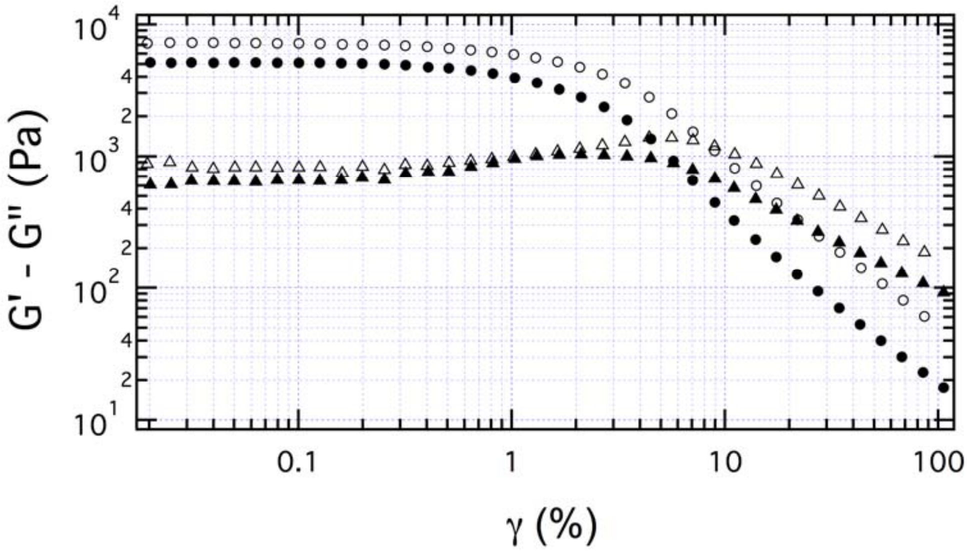

Figure 5: Strain dependence of the storage modulus (G', circles) and loss modulus (G", triangles) of water dispersion of crude apple tissue obtained from fresh (filled symbols) and ripe apple fruit (empty symbols)

The absolute value of the elastic modulus G' in the linear viscoelastic region is ≈ 5,000 Pa, which is roughly one half of the G' value measured for the pure cell wall material (i.e. 10,000 Pa). Gels obtained from the hydration of Crude Apple Tissue shows therefore a lower elasticity character with respect to the re-hydrated

cell wall materials gels. Nonetheless, the self-healing capability is fully maintained (not shown). As the pectic substances are removed in the water-based extraction process, their prevalent role in defining the elastic properties of the gels is proved. Then, the major bear-loading components are cellulose and hemicellulose, whereas water-soluble substances, such as pectins, only contribute to the overall elasticity of the formed network.
When Crude Apple Tissue is extracted form ripe apple flesh, the stress sweep test performed on reconstructed gels delivers a significant increase in the elastic modulus (i.e. ≈9,000 Pa), while the reversible stress softening behaviour is retained. The elastic moduli of CWM and “ripe” CAT gels are therefore remarkably close each other. Understanding of complex phenomena underlying these results (which take into account the physiology of fruit ripening and senescence) is beyond the scope of this work. Nonetheless, our results show that, from a rheological point of view, the Crude Apple Tissue, which was water-extracted from aged and wasted apple fruit, behaves quite similar to hydrated pure cell wall material. The mild extraction process seems therefore to be a sustainable way to produce natural food additives.

4. Conclusion

The mechanical properties of the plant and fruit cell wall play an important role in differentiation and growth of plant and fruit cells. These physiological processes can be defined in mechanical terms as the relaxation of the wall stress. In particular, the self-healing properties of the cell walls components, which has been quantified in this work throughout rheological dynamic testing, can be applied in a technological context and can be considered the backbone for new food additives and/or ingredients. Cell wall materials, or some selected constitutive components, might in fact play a key role as adaptable thickening agent in food formulations, due to their well-known ability to bind a large amount of water. The possible presence of small amounts of solvents residues from the extraction process, which was not quantified in the present work, might be a drawback for the use of these systems in food industry and will be quantified in future studies. Nonetheless it should not influence the rheology of the system. A mild extraction of cell wall materials of from ripe apples beyond their commercial lifetime is a promising process, which needs further investigations.

References

Harris P.J., Smith B.G., 2006, Plant cell walls and cell-wall polysaccharides: structures, properties and uses in food products, International Journal of Food Science & Technology, 41**,** 129-143.

Kunzek H., Müller S., Vetter S., Godeck R., 2002, The significance of physico chemical properties of plant cell wall materials for the development of innovative food products, European Food Research and Technology, 214**,** 361-376.

Mckenna G.B., Narita T., Lequeux F., 2009, Soft colloidal matter: A phenomenological comparison of the aging and mechanical responses with those of molecular glasses, Journal of Rheology (1978-present), 53**,** 489-516.

Ubbink J., Krüger J., 2006, Physical approaches for the delivery of active ingredients in foods, Trends in Food Science & Technology, 17**,** 244-254.

Waldron K.W., Parker M.L., Smith A.C., 2003, Plant cell walls and food quality, Comprehensive Reviews in Food Science and Food Safety, 2**,** 128-146.