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PHYSICAL AND CHEMICAL ATTACHMENT OF PECTINS TO SUBSTRATES : METHODS, CHARACTERISATION AND APPLICATION

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SUMMARY

The plant cell wall is a complex biological matrix in which pectic polysaccharides play an instrumental role in regulating mechanical properties. Nanomechanical studies of single chains hold the promise of enabling the comprehension of fundamental aspects concerning the structural, mechanical and binding properties of pectin at an unprecedented level of molecular detail, using measured single polysaccharide force-extension behavior as a signature. However, before such promise can be fulfilled, a better understanding of the attachment of the polymer under study to the substrates between which it is stretched is required.

Herein, chemoselective methodologies have been developed to covalently couple one end of a pectin chain onto a solid support. Prior to immobilization, pectin fine structure was investigated using accurate and non-invasive infrared spectroscopy. Comparison of experimental results with the predictions of quantum chemical calculations carried out using density functional theory confirmed this technique as an effective tool for the characterization of pectin fine structure. Subsequently, following appropriate functionalization of the support, pectin chains were anchored to polystyrene beads, specifically through their reducing end. These methods were shown to be efficient using IR spectroscopy, once more coupled with quantum chemical calculations, with the formation of specific newly introduced bonds being demonstrated.

Finally, single-molecule force spectroscopy was used to stretch single pectin molecules covalently bonded to substrates using the previously described method applied to glass surfaces. Compared to physisorption, which was also extensively studied, tethering the pectin non-reducing end appeared to increase the average stretch length and improved significantly the probability of stretching a single chain to high forces.

To my joy, my pride, my everything,

my baby boys

ETHAN et RUBEN…

(and the little one coming)…

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Surprisingly (yeah right!), this part is kind of a relief, even if I don't really know where to start. Some many people to thank...

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ABBREVIATIONS

AFM	Atomic Force Microscopy
CI	Confidence Interval
DLS	Dynamic Light Scattering
DFT	Density Functional Theory
DM	Degree of Methyl-esterification
Fig.	Figure
HG	Homogalacturonan
HM	Highly Methyl-esterified
Gal*p*A	Galactopyranosyluronic acid
Kdo	Ketodeoxymannooctulopyranosylonic acid
LM	Lowly Methyl-esterified
NMR	Nuclear Magnetic Resonance
OT	Optical Tweezers
PFM	Peak Fitting Module
PG	Polygalacturonase
PGL	Pectate Lyase
PL	Pectin Lyase
Pka	Dissociation constant
PME	Polymethylesterase
PMG	Polymethylgalacturonase
RA	Reductive Amination
RG I	Rhamnogalacturonan I
RG II	Rhamnogalacturonan II
Rha	Rhamnose
SMFS	Single molecule force spectroscopy
SPM	Scanning Probe Microscopy
STM	Scanning Tunneling Microscope
TF	Thiazolidine Formation